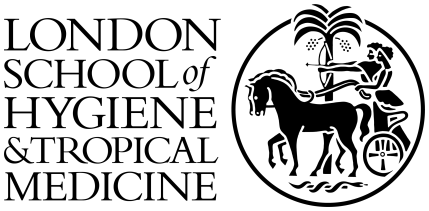

Neurodevelopmental effects of HIV exposure:

A prospective neuroimaging study of uninfected children born to mothers living with HIV

Dr Catherine Scrymgeour-Wedderburn

Thesis submitted in accordance with the requirements for the degree of

Doctor of Philosophy of the University of London

2025

Department of Clinical Research

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London School of Hygiene & Tropical Medicine

Collaborating institutions:

University of Cape Town

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Declaration

I, Catherine Scrymgeour-Wedderburn, declare that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated within the thesis.

Signature: [redacted] **Date:** 12.09.24

Catherine Scrymgeour-Wedderburn

Dedication

To my parents who inspire me to be kind and courageous

To my grandparents who gave me endless love

To my brothers for their support

To my friends for their laughter

To my teachers who guide me

To my students who teach me

To the children who motivate me

To South Africa for showing me the potential to grow, to change, and to hope.

“We can change the world and make it a better place.

It is in your hands to make a difference.”

Nelson Mandela

ABSTRACT

Background: Globally, over one million children are born every year to mothers living with HIV. The successful scale-up of antiretroviral therapy (ART) has reduced vertical HIV transmission, which means that most children are uninfected. Despite avoiding HIV infection, antenatal HIV exposure may still have serious consequences. Understanding the neurodevelopment of children who are HIV-exposed and uninfected (HEU) is therefore of substantial public health importance. Advances in neuroimaging provide insights into biological pathways underlying neurodevelopmental outcomes. The aim of this thesis was to examine the effects of *in utero* exposure to HIV on child brain structure and function.

Methods: The research includes a prospective neurodevelopment and neuroimaging study of HEU and HIV-unexposed (HU) children embedded in the Drakenstein Child Health Study (DCHS). The DCHS is a large population-based birth cohort of over 1000 mother-child pairs in a well-characterised South African community with high HIV prevalence. Validated developmental assessments using the Bayley Scales of Infant and Toddler Development-III and high-resolution structural magnetic resonance imaging (MRI) were undertaken in HEU and HU children from birth to three years. In addition, a conceptual framework and a systematic review and meta-analysis were developed to contextualise the results.

Findings: *Neurodevelopment*: In the DCHS, children who were HEU had two-fold increased risk of language delay compared to HU children at two years; receptive and expressive language scores were associated with maternal CD4 count in pregnancy. Across studies, the systematic review and meta-analysis found HEU children were at risk of poorer expressive language as well as gross motor development relative to HU children, with no consistent effects of maternal ART regimens. Potential HIV-specific and universal mechanisms for impaired development were identified. *Neuroimaging:* The MRI study demonstrated the feasibility of neuroimaging young children without sedation in a sub-Saharan African setting and significant associations between brain structure and neurocognitive function. On average, at age 2-6 weeks, infants who were HEU had smaller total grey matter and basal ganglia volumes compared to HU infants; volumes were associated with maternal CD4. At age 2-3 years, HEU children continued to have lower subcortical brain volumes, notably in the basal ganglia and hippocampus. Regional brain volumes correlated with language scores and were associated with maternal HIV severity. Additionally, HEU children had greater mean cortical thickness in the prefrontal region than HU children, which mediated the relationship between HIV exposure and language outcomes.

Conclusions: This thesis provides novel data that children who are exposed to HIV *in utero* are at risk of impaired language development and altered brain structure in early life. Identified associations between HIV exposure, frontostriatal brain structure, and neurocognition suggest neurobiological pathways may underlie language deficits in children who are HEU. While findings indicate early neurodevelopmental differences, future research will delineate mechanisms and determine long-term implications. Overall, this work expands our understanding of the brain structure-function relationship, provides unique insights into the effects of HIV exposure on the brain during a critical developmental window, and highlights the importance of optimising antenatal HIV care to ensure children thrive.

Thesis Overview

Part I: Introduction, Aim, and Methods

Chapter 1: Introduction

Chapter 2: Study Rationale, Hypothesis, Aim, and Objectives

Chapter 3: Study Protocol and Data Collection Methods

Part II: Neurodevelopment

Chapter 4: Neurodevelopment of HIV-Exposed Uninfected Children in South Africa: Outcomes from an Observational Birth Cohort Study *(research paper)*

Chapter 5: Growth and Neurodevelopment of HIV-Exposed Uninfected Children: a Conceptual Framework *(review paper)*

Chapter 6: Early Neurodevelopment of HIV-Exposed Uninfected Children in the Era of Antiretroviral Therapy: a Systematic Review and Meta-analysis *(research paper)*

Part III: Neuroimaging

Chapter 7: Neuroimaging Young Children and Associations with Neurocognitive Development in a South African Birth Cohort Study *(research paper)*

Chapter 8*:* Early Structural Brain Development in Infants Exposed to HIV and Antiretroviral Drugs *In Utero* in a South African Birth Cohort *(research paper)*

Chapter 9: Subcortical Brain Volumes and Neurocognitive Function in Children with Perinatal HIV Exposure: A Population-based Cohort Study in South Africa *(research paper)*

Chapter 10: Association of *In Utero* HIV Exposure with Child Brain Structure and Language Development: A South African Birth Cohort Study *(research paper)*

Part IV: General Discussion and Implications

Chapter 11: Discussion and Overall Conclusions

Chapter 12: Clinical, Policy, and Research Implications

Preface

This thesis is presented as a 'Research Paper Style Thesis' in accordance with submission guidance provided by the London School of Hygiene & Tropical Medicine. Seven chapters comprise papers that have been published, the introduction and discussion include material adapted from a published book chapter, and there are two published papers included in the appendices. The chapters prepared as papers are indicated in the ***Table of Contents*** of the thesis. All papers are published under the name Catherine J Wedderburn. Publication details and acknowledgement of co-author contributions are included on the individual cover sheets for each paper. In view of the differing requirements of the journals in which the work has been published, there is by necessity some repetition of material, notably in the methods sections, and variation in the formatting of these chapters. Additionally, accepted nomenclature has changed over time so throughout the PhD thesis the terminology may differ according to journal preference. I acknowledge that this may not always align with current preferred terminology. The remainder of the thesis is comprised of 'linking material' and includes an overall introduction and forewords to each chapter to connect the story, as well as a discussion and appendices.

All material within this thesis was written by Catherine Scrymgeour-Wedderburn.

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Acronyms and Abbreviations

ANC	Antenatal clinic
ARV	Antiretroviral drug
ART	Antiretroviral therapy
ASD	Autism spectrum disorder
BSID-III	Bayley Scales of Infant and Toddler Development, 3rd Edition
CMV	Cytomegalovirus
CNS	Central Nervous System
CUBIC	Cape Universities Body Imaging Centre
DCHS	Drakenstein Child Health Study
DTI	Diffusion Tensor Imaging
FA	Fractional anisotropy
fMRI	functional Magnetic Resonance Imaging
FSL	fMRI of the brain (fMRIB) software library
HEU	HIV-exposed uninfected
HIC	High-income countries
HIV	Human immunodeficiency virus
HU	HIV-unexposed (uninfected)
ICU	Intensive Care Unit
KABC	Kaufman Assessment Battery for Children
LMIC	Low and middle-income countries
LSHTM	London School of Hygiene & Tropical Medicine
mOFC	Medial orbitofrontal cortex
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
PMTCT	Prevention of mother-to-child transmission
RNA	Ribonucleic acid
ROI	Region of interest
SSA	Sub-Saharan Africa
TBSS	Tract-based spatial statistics
UCLA	University of California, Los Angeles
UCT	University of Cape Town
VL	Viral Load
WHO	World Health Organization

Definitions

The below definitions are adapted from the book chapter noted in the Introduction cover sheet: Wedderburn CJ, Yeung S, Donald KA. Neurodevelopment of children who are HIV-exposed and uninfected. In: Recent Advances in the Neurological and Neurodevelopmental Impact of HIV. Editors: Amina Abubakar, Kirsten A Donald, Jo M Wilmshurst, Charles R Newton. Mac Keith Press 2023.

Children who are HIV-exposed and uninfected (HEU): Children born HIV-free to women living with HIV. Children born to mothers living with HIV may be exposed to HIV *in utero,* during the birth process and through breastfeeding in the postnatal period. In areas where ART has been scaled up, many HIV-exposed children have also been exposed to ART as well as other factors associated with being born into a family affected by HIV. Preferred nomenclature has changed over time so throughout the thesis the terminology may differ according to journal preference. As the literature is evolving, I acknowledge that the terminology may continue to change.

Children who are HIV-unexposed (HU): Children born to mothers without HIV infection.

Antiretroviral therapy (ART): The combination of antiretroviral drugs given to treat HIV infection.

Neurodevelopment: Neurodevelopment describes the development of the nervous system and the brain's neural networks responsible for performance and function. In the early years, these functions include cognitive, language, and motor development as well as behaviour. For the purpose of this thesis, I use the term neurodevelopment in terms of clinical outcomes to refer to early child development including cognitive, motor, and language outcomes.

Neuroanatomy: This describes the structure and organisation of the nervous system. In this thesis, the focus is on the brain including the cortical and subcortical regions.

Acknowledgements

This thesis is the culmination of support from many people who have shaped my journey. I am forever grateful to all of you.

I would like to first thank my incredible supervisors Professor Kirsty Donald, Professor Shunmay Yeung, and Professor Diana Gibb for their constant encouragement, patience and input into this project. Your passion for improving child lives through both the academic and clinical spheres is truly inspiring. Kirsty, your kindness and grace shine through in everything you do and I learn from it every day. You are an inspiration to everyone around you, and I feel so lucky to work with you. Shunmay, I am immensely grateful that you stepped in to help when I most needed it and for being wonderful, giving me the space to grow whilst always being there with a smile and support. Di, thank you for your wise guidance in everything. I admire you and your work so much and hope that one day I will contribute as much to the world as you have done.

I am indebted to Professor Heather Zar and Professor Dan Stein for their invaluable support and advice and for the privilege of working with the Drakenstein Child Health Study. I have learnt so much from you both and thank you for welcoming me into the unique research community that you have built and for your wisdom along the way.

I would also like to make a special mention to Assoc. Professor Andrea Rehman. I could not have achieved this without you. You went so above and beyond what was required, teaching me statistics and always being a voice of reason; thank you for everything.

Most importantly, the work would not have been possible without the contributions of all the mothers, children, and families participating in the Drakenstein Child Health Study. It has been such a privilege and joy to spend time and get to know the mothers and children through the hours spent in the MRI scanner. I would like to express my heartfelt gratitude to them all and hope that this work may help the lives of others going forward.

Huge thanks to the wider Drakenstein Child Health Study team at the clinics and Paarl hospital and the REACH office for welcoming me, and particularly to Whitney Barnett, Tiffany Burd, Nadia Hoffman, and Attie Stadler for being fountains of knowledge and friends, and to the site nurses and fieldworkers who work so tirelessly and care so deeply. I am incredibly grateful to the neuroimaging team and CUBIC radiographers (photo in the methods) for all the late nights we worked to get the children to sleep through MRI scans!

And to Annerine Roos, Nynke Groenewold, Jean-Paul Fouche, and Sivenesi Subramoney for making working together on this research so enjoyable. It has also been a great honour to develop connections and learn from Professor Katherine Narr and Shantanu Joshi at UCLA, and to visit their lab there.

Thank you to my other colleagues, teachers, and students for their help, advice, and friendship and everyone I have met on the way who has contributed to expanding my interest and work in this area, particularly Professor Andrew Prendergast and Dr Ceri Evans. I am grateful to Dr Cally Tan and Dr Jonathan Underwood for all their advice and recommendations in the early parts of my thesis, and for being on my advisory committee. I would also like to acknowledge everyone at LSHTM who helped me to manage the study, in particular, Professor David Mabey and Tamara Hurst. Finally, this project would not have happened without the initial support and guidance of Professor Stephen Lawn, who inspired and encouraged me in all my work. I miss his mentorship dearly and often wonder on his advice, but I thank Joy for being such a wonderful mentor in his absence.

I thank the Wellcome Trust for the Research Training Fellowship which has allowed me to do this work and changed the course of my life.

And now, my family – I know the road has been long, but it has been a great journey. Mum and Dad, nothing I can write here will ever do justice to the love, support, and encouragement that you have given me. Thank you from the very bottom of my heart, without you this would not have been possible. To Charles, Alexander, Rowan, Rosie, and all my amazing friends - thank you for being there through it all, for filling my life with joy and laughter, and for making this journey so much brighter - each of you is truly special to me and I am deeply grateful to have you in my life.

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Name	Position/Institution	Contribution
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Detailed outline of thesis

This thesis comprises a combination of research articles and explanatory linking material. In total, there are 12 chapters. A detailed outline of the thesis is provided below.

PART I: Introduction, Aim, and Methods has three chapters.

Chapter 1: General introduction with an overview of HIV epidemiology. This is followed by a summary of evidence on the neurodevelopment and neuroimaging outcomes of children who are HEU at the time of the PhD onset, adapted from a published book chapter:

Wedderburn CJ, Yeung S, Donald KA. Neurodevelopment of children who are HIV-exposed uninfected. In: Recent Advances in the Neurological and Neurodevelopmental Impact of HIV. Editors: Abubakar A, Donald KA, Wilmshurst JN, Newton CR. Mac Keith Press 2023.

Chapter 2: This chapter outlines the scientific rationale for the research conducted along with the hypothesis, aim, and objectives.

Chapter 3: This chapter sets out the methods used and describes the Drakenstein Child Health Study (DCHS) population-based cohort study site and participants, along with the methodology, data collection, and ethical considerations.

PART II: Neurodevelopment has three chapters; all published in peer-reviewed journals. This section addresses objective 1.

Chapter 4: This research paper describes the early neurodevelopmental outcomes of HEU compared to HU children in the DCHS. While no differences were seen at six months, on average children who were HEU were found to have impaired language development with increased language delay at two years of age compared to HU children. Maternal immunosuppression was associated with poorer language outcomes.

Wedderburn CJ, Yeung S, Rehman AM, *et al*. Neurodevelopment of HIV-exposed uninfected children in South Africa: outcomes from an observational birth cohort study. *Lancet Child Adolesc Health.* 2019; 3(11): 803-13.

Chapter 5: This review paper outlines a conceptual framework for how exposure to HIV may lead to adverse development. The framework proposes that HEU children are affected (i) indirectly, through the augmentation of universal risk factors underlying poor development, and (ii) directly through HIV/ART-specific pathways, which ultimately may converge through common pathogenic mechanisms.

Wedderburn CJ, Evans C, Yeung S, Gibb DM, Donald KA, Prendergast AJ. Growth and Neurodevelopment of HIV-Exposed Uninfected Children: a Conceptual Framework. *Curr HIV/AIDS Rep.* 2019; 16(6): 501-13.

Chapter 6: This systematic review and meta-analysis paper compares the neurodevelopment of HEU and HU children. Overall, HEU children were found to be at risk of subtle impairments in expressive language and gross motor development by age two years. While there was no consistent effect of maternal ART regimens analysed, evidence was scarce. The paper highlights the need for high-quality longitudinal and neuroimaging studies to assess the neurodevelopmental trajectories of HEU children, setting the scene for the neuroimaging.

Wedderburn CJ, Weldon E, Bertran-Cobo C, et *al.* Early neurodevelopment of HIV-exposed uninfected children in the era of antiretroviral therapy: a systematic review and meta-analysis. *Lancet Child Adolesc Health*. 2022; 6(6): 393-408.

PART III: Neuroimaging has four chapters; all are published in peer-reviewed journals. This section addresses objectives 2 and 3.

Chapter 7: This research paper presents the DCHS neuroimaging methods and associations between brain structure and function. MRI is an indispensable tool for investigating brain development in young children and the neurobiological mechanisms underlying developmental risk and resilience. However, paediatric MRI imaging is challenging due to motion sensitivity. This paper demonstrates the feasibility of neuroimaging children during natural sleep in sub-Saharan Africa. The findings indicate that dynamic morphological changes in heteromodal association regions are associated with neurocognitive development.

Wedderburn CJ, Subramoney S, Yeung S, *et al*. Neuroimaging young children and associations with neurocognitive development in a South African birth cohort study. *NeuroImage*. 2020; 219: 116846.

Chapter 8: This research paper compares structural brain development of infants who were HEU and HU at 2-6 weeks of age. Smaller caudate (basal ganglia nuclei) and total grey matter volumes were found in HEU compared to HU infants. Maternal immunosuppression was associated with reduced volumes. These findings suggest that antenatal HIV exposure may impact early structural brain development and improved antenatal HIV management may optimise neurodevelopmental outcomes.

Wedderburn CJ, Groenewold NA, Roos A, *et al*. Early structural brain development in infants exposed to HIV and antiretroviral therapy in utero in a South African birth cohort. *J Int AIDS Soc*. 2022; 25(1): e25863.

Chapter 9: This research paper presents the 2-3 year old subcortical brain imaging findings. Similar to the findings in infancy, smaller subcortical volumes were detected in children who were HEU, notably in the basal ganglia region (putamen nuclei). Additionally, children who were HEU had smaller volumes of the hippocampus, a region known to be vulnerable to early-life exposures. Volumes were associated with language development and maternal CD4 count and viral load in pregnancy. These findings suggest that *in utero* HIV exposure may affect early subcortical brain development with enduring impact and functional implications.

Wedderburn CJ, Yeung S, Groenewold NA, et al. Subcortical brain volumes and neurocognitive function in children with perinatal HIV exposure: a population-based cohort study in South Africa. *Open Forum Infect Dis*. 2024;11(7):ofae317

Chapter 10: This research paper describes the cortical neuroimaging findings. Children who were HEU were found to have altered patterns of cortical structure in early life compared to HU children, with greater cortical thickness in the medial orbitofrontal region which mediated the relationship between HIV exposure and poor language outcomes. The findings indicate that differences in cortical thickness development in HEU children may be a pathway leading to language impairment.

Wedderburn CJ, Yeung S, Subramoney S, *et al*. Association of *in utero* HIV exposure with child brain structure and language development: a South African birth cohort study. *BMC Med*. 2024;22(1):129

PART IV: General Discussion and Implications has two chapters

Chapters 11: This discussion chapter provides a detailed interpretation of the main findings in the preceding chapters in light of existing literature. Strengths and limitations of the thesis as a whole are discussed.

Chapter 12: The final chapter discusses implications of this work for clinical practice and public health policy, and includes suggestions for future research steps. This chapter uses the work of the whole thesis and includes an overall conclusion.

APPENDICES

This includes additional details on the methodology, supplementary information of the core chapters, and two additional published papers:

Green F, du Plooy C, Rehman AM… Wedderburn CJ. Language outcomes of preschool children who are HIV-exposed uninfected: an analysis of a South African cohort. *PLoS ONE* 2024;19(4):e0297471

Bertran-Cobo C^, Wedderburn CJ^, Robertson FC, *et al.* A neurometabolic patterns of elevated myo-inositol in children who are HIV-exposed and uninfected: a South African birth cohort study (^=co-first authors). *Front Immunol* 2022;13:800273

Part I

Introduction, Aim, and Methods

1

Chapter 1: Introduction

Introduction

Summary

Human Immunodeficiency Virus (HIV) is a global health priority. There are 39 million people living with HIV worldwide, and women of reproductive age represent a large proportion of new infections. However, the successful scale up of antiretroviral therapy (ART) in pregnancy has resulted in a substantial decline in the vertical transmission of HIV. This means that most children born to women living with HIV will remain HIV-free. Every year over 1 million children are born who are HIV-exposed and uninfected (HEU), and in some countries HEU children represent over 20% of annual births. As the HIV epidemic shifts with the expanding use of ART, the health outcomes of children who are HEU are becoming of critical importance. Previous studies have suggested that, despite avoiding infection, exposure to HIV may have serious consequences for uninfected children. However, the evidence for child neurodevelopmental outcomes is inconclusive. Early neurodevelopment lays the foundation for future academic and economic outcomes, therefore, any neurodevelopmental impairment resulting from HIV exposure will have a substantial impact at both the individual and wider population level. Neuroimaging may be used as a tool to understand brain development and biological pathways that underlie neurodevelopmental outcomes and deficits. The following chapter provides (i) an overview of HIV epidemiology and children who are HEU; (ii) a description of the literature on HIV exposure and child neurodevelopment at the study onset; (iii) a framework for considering risk and protective factors for development in children who are HEU; and (iv) an introduction to neuroimaging, brain development, and the role of magnetic resonance imaging (MRI) in understanding HEU child neurodevelopment.

Parts of the text and the two figures in this section are adapted from a published book chapter.

Book chapter: Wedderburn CJ, Yeung S, Donald KA. Neurodevelopment of children who are HIV-exposed uninfected. In: Recent Advances in the Neurological and Neurodevelopmental Impact of HIV. Editors: Amina Abubakar, Kirsten A Donald, Jo M Wilmshurst, Charles R Newton. Mac Keith Press 2023.

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	383999	**Title**	Dr
First Name(s)	Catherine		
Surname/Family Name	Scrymgeour-Wedderburn		
Thesis Title	Neurodevelopmental effects of HIV exposure: a prospective neuroimaging study of uninfected children born to mothers living with HIV		
Primary Supervisor	Professor Shunmay Yeung		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Book chapter: Wedderburn CJ, Yeung S, Donald KA. Neurodevelopment of children who are HIV-exposed uninfected. In: Recent Advances in the Neurological and Neurodevelopmental Impact of HIV. Editors: Abubakar A, Donald KA, Wilmshurst J, Newton C. Mac Keith Press 2023		
When was the work published?	2023		
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For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceived, researched, and wrote the first draft with input from my co-authors, and updated and edited the manuscript following peer review.

SECTION E

Student Signature	[redacted]
Date	12.09.24

Supervisor Signature	[redacted]
Date	12.09.24

1.1 HIV: A global health priority

Human immunodeficiency virus (HIV) is a major public health issue; while great progress has been made in the last four decades, new challenges have emerged. HIV is a retrovirus that targets the body's immune system. Without treatment, the virus eventually causes acquired immunodeficiency syndrome (AIDS) and death. HIV spread widely in the late 1900s to become a global health priority. In 2001, the United Nations convened the first special session on HIV/AIDS which followed recognition of the pandemic as a threat to global peace and security, the first time for a health issue. The discovery and scale up of antiretroviral drugs (ARVs) since the 1980s has substantially reduced the number of new HIV infections and transformed the disease into a chronic lifelong condition, however, the prevalence continues to rise. Across the world, 39 million people are living with HIV, and two thirds reside in Africa.[1]

1.1.1 HIV pathogenesis and antiretroviral therapy

HIV targets the immune system CD4+ T-lymphocyte cells with potentially devastating effects.[2] In the acute stage of the infection, there is dissemination of the virus with a peak in viral load and a drop in CD4+ T-lymphocytes. Without treatment, this is followed by chronic infection and clinical latency over many years where there is a slow decline in CD4+ T-lymphocyte numbers and an increase in HIV ribonucleic acid (RNA) copies. During this time, chronic immune activation is a key feature of HIV pathogenesis. Eventually the ongoing evolution of HIV overcomes the immune system neutralising antibody responses leading to immunodeficiency. The advanced stage of HIV is characterised by symptomatic infection and vulnerability to opportunistic infections, known as AIDS.[2,3] ARVs prevent replication of HIV and halt the pathogenesis of the virus, altering the clinical course to maintain health and survival. This is achieved by targeting steps in the viral replication cycle including reverse transcription (nucleoside reverse transcriptase inhibitors and non-nucleoside reverse transcriptase inhibitors), fusion (fusion inhibitors), integration (integrase inhibitors), and virus assembly (protease inhibitors). Combination antiretroviral therapy (ART) is the cornerstone of HIV management alongside effective prevention, diagnosis, and care. ART suppresses viral replication activity and slows the emergence of resistance, thereby decreasing transmission. The global 95-95-95 goals state that by 2025, 95% of all people living with HIV should have a diagnosis, 95% of those should be taking ART, and 95% of those on ART should have a suppressed viral load.[4] Currently there is no cure for HIV and even with ART the virus persists in cellular and/or anatomical reservoirs.[5]

1.1.2 HIV disease severity and transmission

HIV disease severity may be assessed through biomarkers including the CD4 cell count and HIV RNA viral load.[6-8] These prognostic markers are able to predict the risk of disease progression and transmission, with worsening disease associated with lower CD4 cell count and higher HIV viral load. They are correlated but independent;[8] in the acute stage, viral load plays a greater role in prognosis, whereas later in the disease process, the CD4 cell count is more predictive. Additionally, clinical symptoms are useful indicators of disease status.

Transmission of HIV occurs via contact with blood or other bodily fluids, including breast milk, carrying the HIV virus.[9] The majority of transmission occurs in adults, which may occur via sexual transmission or parenterally through intravenous drug use or blood transfusions.[10] HIV disproportionately affects women,[2] in particular, adolescent girls and young women of reproductive age who account for 60% of new HIV infections among 15-24 years olds globally.[11] In sub-Saharan Africa (SSA), adolescent girls and young women make up over 77% of new infections in that age group.[12] The most common mode of child infection is through vertical transmission which may occur across the placenta during pregnancy, in the birth process, or through breastfeeding.[13]

1.1.3 HIV in pregnancy and prevention of vertical transmission

Worldwide, an estimated 1.3 million pregnant women are living with HIV.[14,15] Without ART or other interventions, a high proportion of children born to mothers living with HIV will acquire HIV, with vertical transmission rates ranging from 15-45% depending on the duration of breastfeeding.[16] However, the successful scale up of ART in pregnancy has resulted in a substantial decline in the vertical transmission of HIV and the number of children born with HIV in areas where programmes are implemented.[1] ART in pregnancy suppresses the virus, reducing maternal HIV viral load, which is key to limiting vertical transmission risk.[17] Vertical transmission may be reduced to <1% by maternal ART.[18]

In 2010, the WHO recommended that pregnant women living with HIV should start ART if required for their own health or short-term ARV prophylaxis (zidovudine) if they were not eligible for ART. In 2013, the guidelines changed so that initiation of ART was no longer determined by a women's health status, instead ART should be given to all pregnant women living with HIV and continued for the duration of breastfeeding or for life, depending on country guidance. Since 2015, the WHO has recommended lifelong ART, regardless of WHO clinical staging or CD4 cell count, for all people with confirmed HIV infection, to be initiated at the earliest opportunity.[19] ART continues to be scaled up across the world, and in 2022 it was

estimated that 82% of pregnant women with HIV received ARVs for prevention of mother-to-child transmission of HIV (PMTCT).[1] Therefore, most children born to mothers living with HIV in the current ART era now remain HIV-free.[1,14,20]

1.2 Children who are HIV-exposed uninfected: A growing population

Globally, there are an estimated 16 million children who are HIV-exposed and uninfected (HEU). Continuing high antenatal HIV prevalence in many countries means that over one million infants are born every year with *in utero* HIV exposure who will remain HIV-free; the child HEU population is therefore increasing rapidly (Figure 1.1).[1] Over 90% of HEU children are living in SSA,[14] and in some countries, including South Africa, Lesotho, Botswana and Eswatini, more than one in five children are born HEU.[1] Any problems resulting from HIV exposure will thus have a substantial impact at both the individual and national level in this region.[21] As the HIV epidemic treatment landscape shifts, the health outcomes of children who are HEU are now recognised to be of critical importance.

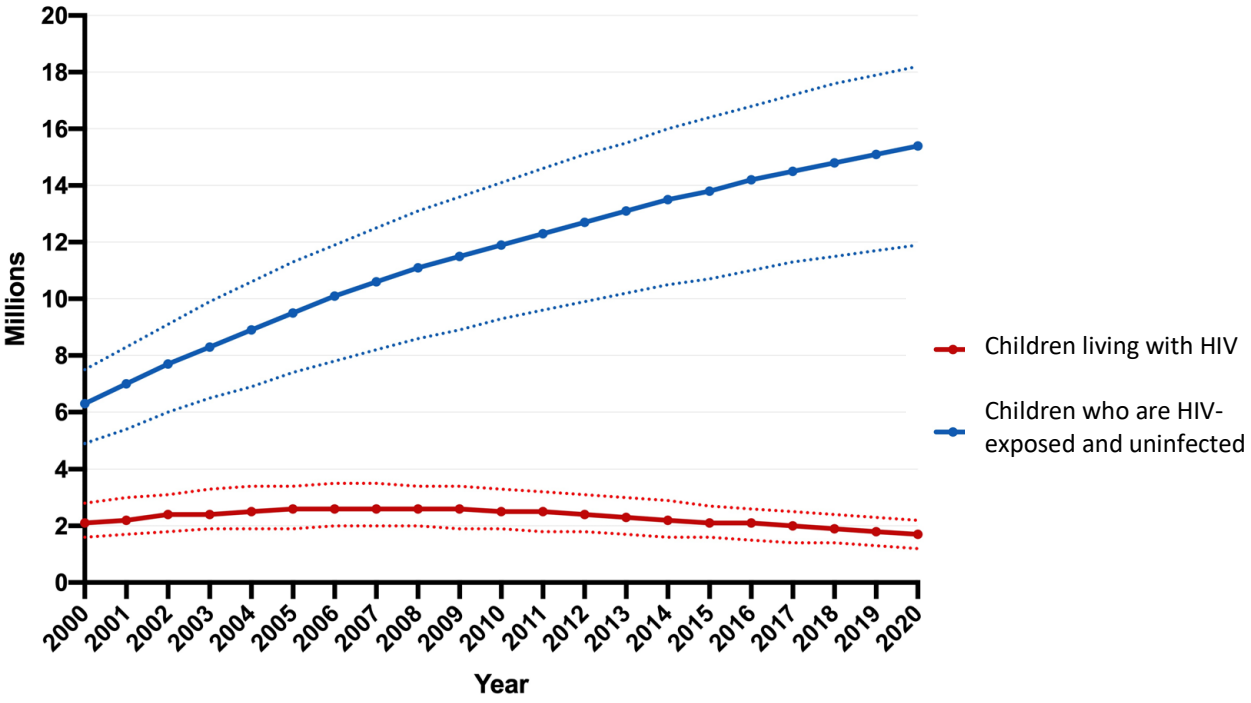

Figure 1.1: Children (aged 0-14 years) who are HIV-exposed and uninfected and children living with HIV: global numbers, 2000-2020. Data from UNAIDS.[1] Figure taken from the book chapter Wedderburn CJ, Yeung S, Donald KA. Neurodevelopment of children who are HIV-exposed uninfected.

1.2.1 HIV exposure and child health

Extensive literature documents the detrimental effects of HIV infection in children living with the virus, particularly on the central nervous system (CNS).[22] While early ART ameliorates the

effects of HIV, reports of continued neurobehavioural deficits in children living with HIV remain.[22,23] In parallel, growing research now suggests that, despite avoiding HIV infection, *in utero* exposure to HIV and/or ART can have potentially serious consequences for children who remain HIV-free.

Emerging evidence indicates that children who are HEU have increased mortality and morbidity, growth failure, and immunological deficits compared to HIV-unexposed (HU) children.[21,24] Although many of these studies are from the pre-ART era, recent studies support these same conclusions.[25,26] Studies from across the world have found that children who are HEU have a higher mortality rate than HU children,[27,28] notably in the first 12 months of life. While reports vary across countries, early-life mortality in HEU children is estimated to be double that of HU children.[29,30] Other studies have found infants who are HEU have increased rates of hospitalisation.[31] The increase in hospitalisation and mortality appear to be primarily due to infectious causes, and a report from South Africa showed that infants who are HEU have increased risk of contracting more severe and unusual diseases early in life.[32] This is likely as a result of alterations in their immune systems when compared to HU infants, with reports of immune deficits and differences in cell-mediated immunity and antibody levels in HEU children.[33] Differences in growth have also been observed, including shorter length at birth, increased stunting in early life, and reduced head circumference when compared to HU children,[34-36] suggesting that HIV-related changes occur early in the developmental process. Stunting is recognised to be a proxy for early child development.[37] Further, head circumference has been correlated with brain growth, which occurs most rapidly in the first few years of life. Given brain growth is closely linked to neurodevelopment,[38] this raises concerns over the neurodevelopmental outcomes of children who are HEU.

1.2.2 HIV exposure and neurodevelopment

Approximately 250 million (43%) children under the age of five years old in low- and middle-income countries (LMICs) are at risk of not reaching their developmental potential, as assessed through stunting and extreme poverty from all causes.[39,40] SSA, where the majority of people living with HIV reside, has the highest prevalence of at-risk children (66% of children under five years of age).[39] Although the effects of child HIV infection have been widely described, the impact of HIV exposure without infection on neurodevelopment remains uncertain. However, there is rising concern that the neurodevelopment of children who are HEU may be adversely affected when compared to their unexposed peers.

To review the literature, relevant publications were identified through a literature search carried out in electronic databases (PubMed and SCOPUS) at the start of the thesis to provide a

background to the study. Included systematic reviews and meta-analyses are summarised below and listed in a table in Appendix II.* Overall, evidence from systematic reviews and meta-analyses suggested that HEU children may have impaired neurodevelopment compared to HU children in the first few years of life, although studies had limitations. A network meta-analysis reported worse cognitive (Mental Development Index) and motor (Psychomotor Developmental Index) function in HEU children compared to HU children in studies using the Bayley Scales of Infant and Toddler Development.[41] However, there were no high quality studies from outside of the USA. A prior systematic review reported lower performance of HEU children compared to HU across psychological measures in 7/11 studies.[42] Similarly, other literature reviews have reported a modest but significant effect on neurodevelopment of children exposed to HIV and ART,[35] with delays in cognition, motor skills, and language expression. However, there were only a few studies of HEU children from Africa and Asia.[43] Finally, one systematic review of studies in SSA from 2016 found no difference in the preschool years between HEU and HU children,[44] although the review concluded that more evidence is needed to understand this population as most studies to date had substantial limitations.

On examination of individual study findings, differences were evident across contexts and ages. The epidemiology of HIV infection varies across settings - in high income countries (HICs) such as Europe and the USA, adult infections are often found concentrated in groups with higher substance use, compared to the more generalised population-level epidemic seen in SSA. As such, the HIV/AIDS pandemic is made up of multiple separate epidemics which need to be considered. Studies from LMICs generally reported worse outcomes for children who are HEU compared to HU with an emerging pattern of developmental delay in HEU children impacting acquisition of early developmental milestones and/or later cognitive outcomes.[42,45] Domains of neurodevelopment reported to be affected included: language, motor function, behaviour, and cognition. The Pediatric Randomized Early versus Deferred Initiation in Cambodia and Thailand (PREDICT) cohort study from Cambodia and Thailand found HEU children aged 2-12 years had lower IQ and delays in language and fine motor development compared to HU children.[46] Language delay was also reported in the Democratic Republic of Congo in children aged 18-72 months.[47,48] However, many of these studies included populations spanning prior to ART. A South African study that examined infants born to mothers taking triple ART reported an increased risk of delayed motor and cognitive development at 12 months in HEU compared to HU children.[49] Another study in Cameroon found children who are HEU aged 4-9 years had lower cognitive scores than HU children, but highlighted the importance of contextual factors.[50] In contrast, other studies found similar development between HEU children exposed to ART

* Appendix II also highlights recent publications that are incorporated into the thesis Discussion.

compared to HU children, including a large study from Uganda and Malawi,[51] and research from Canada found differences in neurodevelopment between HEU and HU children were not sustained after controlling for maternal substance use.[52] Others from the USA reported similar neurodevelopmental outcomes in children under 2 years,[53] although reports of negative cognitive and behavioural outcomes at older ages have emerged.

In summary, evidence at the start of this thesis suggested that HEU children may be at risk of experiencing poorer neurodevelopmental outcomes compared to HU children. However, data from the current ART era were lacking and findings were inconsistent. There was heterogeneity between studies in design, tools, age ranges, population demographics, breastfeeding and ART usage and the quality of studies also varied, making it difficult to draw firm conclusions from existing data. Common methodological limitations identified across individual studies included a lack of appropriate comparative groups and longitudinal data; small sample sizes limiting power to detect subtle differences; and inadequate consideration of potentially confounding environmental factors. Over the past decade international guidelines have changed considerably and although mothers are remaining healthier with ART, their children now have additional exposures. The impact of HIV on child neurodevelopment in the current era of lifelong ART therefore remains to be determined.

1.3 HIV exposure and neurodevelopment: Potential mechanisms

There is a growing appreciation of the impact of early fetal exposures, such as maternal infection, on child brain outcomes.[54-56] Possible mechanisms by which HIV exposure may affect neurodevelopment include chronic maternal inflammation and immune activation, a direct effect on fetal immunity, toxicity from ART exposure, as well as family and environment factors that may be altered by living in an HIV-affected household (Figure 1.2).

1.3.1 HIV exposure and immunity

From a biological perspective, it may be hypothesised that HIV exposure can influence child neurodevelopment through various pathways from direct exposure to HIV virions and proteins through to immune system alterations. In women living with HIV, immune activation and chronic systemic inflammation related to HIV may impact the uterine environment and fetal development, even without fetal infection.[57] Similarly, in children, HIV exposure has been demonstrated to cause immune activation and inflammation,[58] which may lead to the release of neurotoxins that cause neuronal adaptation and impaired neural and brain network function.[59] The interactions between the nervous and immune systems are complex, and the immune system plays a substantial role in brain development.[60] A number of studies have shown

immune system abnormalities among HEU children including a cell profile that appears different to both HU children and children living with HIV for T-cell activation and altered lymphocyte subsets.[33] The cause of these differences is unclear and many of these immunological changes may result from exposure to HIV virions and proteins. Other studies have described both upregulated and downregulated cytokines in HEU children compared to HU children.[33,61] These cytokines are involved in various aspects of neural development,[62] and certain brain maturation processes such as cell migration and axonal growth may be vulnerable to these immune changes at different stages of development.[62,63] In support of a biological hypothesis, HIV disease severity in mothers shows a distinct link to infant health outcomes,[64] and one study found cumulative maternal HIV viraemia during pregnancy was associated with higher odds of motor and expressive language delay in HEU children at 12 months.[65]

1.3.2 ART exposure

Maternal ART is crucial for preventing vertical transmission of HIV and maintaining the health of mothers, however, the potential impact of *in utero* exposure to ARVs on brain development is relatively undocumented. Exposure to ARVs may occur via the placenta, through breast milk, or directly as prophylaxis to the infant and may cause a range of side effects. Over the past decade, the scale-up of ART has led to improved maternal health and prevented vertical HIV transmission. However, it also means that more infants are being exposed to ART *in utero,* as prophylaxis after birth and during breastfeeding, and more women are conceiving on ART. Within the current ART era, there has been an increased focus on whether there are unintended consequences associated with exposure to ARVs *in utero*.[35] Increased preterm delivery, low birth weight, being small-for-gestational age, and metabolic abnormalities have been documented.[66-68] Data from an early trial of zidovudine did not find any long-term neurological consequences (PACTG 076 trial).[69] Conversely, studies in France raised concerns about mitochondrial abnormalities with exposure to nucleoside reverse transcriptase inhibitors (NRTIs).[70,71]

More recently, the Surveillance Monitoring for ART Toxicities in HEU children (SMARTT) study was established by the Pediatric HIV/AIDS Cohort Study (PHACS) in the USA to give insight into the effect of exposure to ARVs in HEU children. Generally the results have been reassuring,[72] although the study showed a few differences in outcomes in HEU children less than two years of age associated with specific ARVs. Study reports found increased late language development in HEU children at 12 months,[73] and an association between antenatal exposure to atazanavir and delayed language.[53] However, later papers from the same cohort did not replicate the association at 24 months, suggesting the effects may be transient.[73,74] Separately, didanosine and stavudine have been associated with adverse child

neurodevelopment,[72] although other ARVs that are now used more commonly have not been found to have major adverse impacts on neurodevelopment at this stage.[53] While concerns for neural tube defects with exposure to ARVs at conception, including dolutegravir, have been raised,[75] these effects have since attenuated with expanding numbers,[76-78] and other studies have shown health benefits with earlier ART initiation.[79] Overall, continued data surveillance on the safety of ARVs and treatment combinations is needed.[58]

1.3.3 Family context

There are many other factors that are known to play a role in influencing the early development of HEU children. The ecological concept of child development as an ongoing biological and psychological process, influenced by the wider environment including family, school, and society, has gained particular recognition in developmental literature.[80] This supports the idea that the family and social context have a critical influence on long-term neurodevelopmental outcomes. This includes the parent-child relationship and parental physical and psychosocial health. Children born into families affected by HIV may face multiple social and environmental adversities that differ from children unaffected by HIV. In the context of HIV, family and the parent-child relationship may be impacted through physical illness, parental death, parental mental illness, and strain from emotional, financial, and social pressures including stigma.[81] In particular, depression is well-documented in women with HIV and may affect fetal development, as well as maternal-child interaction.[82] Parental physical health and corresponding caregiving ability may also be affected by HIV, and this has wide-ranging implications for child outcomes. Finally, it is becoming evident that other factors such as genetic vulnerabilities and epigenetic programming play important roles.

1.3.4 Environment, social, and economic determinants

The environment, social, and economic ('structural') determinants of health have been extensively documented to influence child development, including housing, nutrition, security, sanitation, and access to healthcare, with poverty as a route cause of many of these factors.[24,37,39] These may impact neurodevelopment through a myriad of pathways, with far-reaching effects,[21] and negative associations with brain volume and academic performance have been found.[83] Environmental exposure to other infections and toxins puts children at higher risk for poor outcomes. Children who are HEU may be exposed to more infections early in life through living with family members with HIV, notably cytomegalovirus (CMV) infection which has been linked to developmental delays.[84,85] Separately, literature demonstrates that maternal alcohol and other substance use, particularly during pregnancy, may have a negative impact on child neurodevelopment.[86,87] HIV exposure may also influence infant feeding and nutrition,

which play key roles in child health outcomes providing both protection against infectious diseases and long-term health benefits.[88] Of note, breastfeeding advice to mothers with HIV has changed over time, reflecting the evolving understanding of vertical transmission risks and benefits of breastfeeding on cognitive outcomes and child brain development.[88,89]

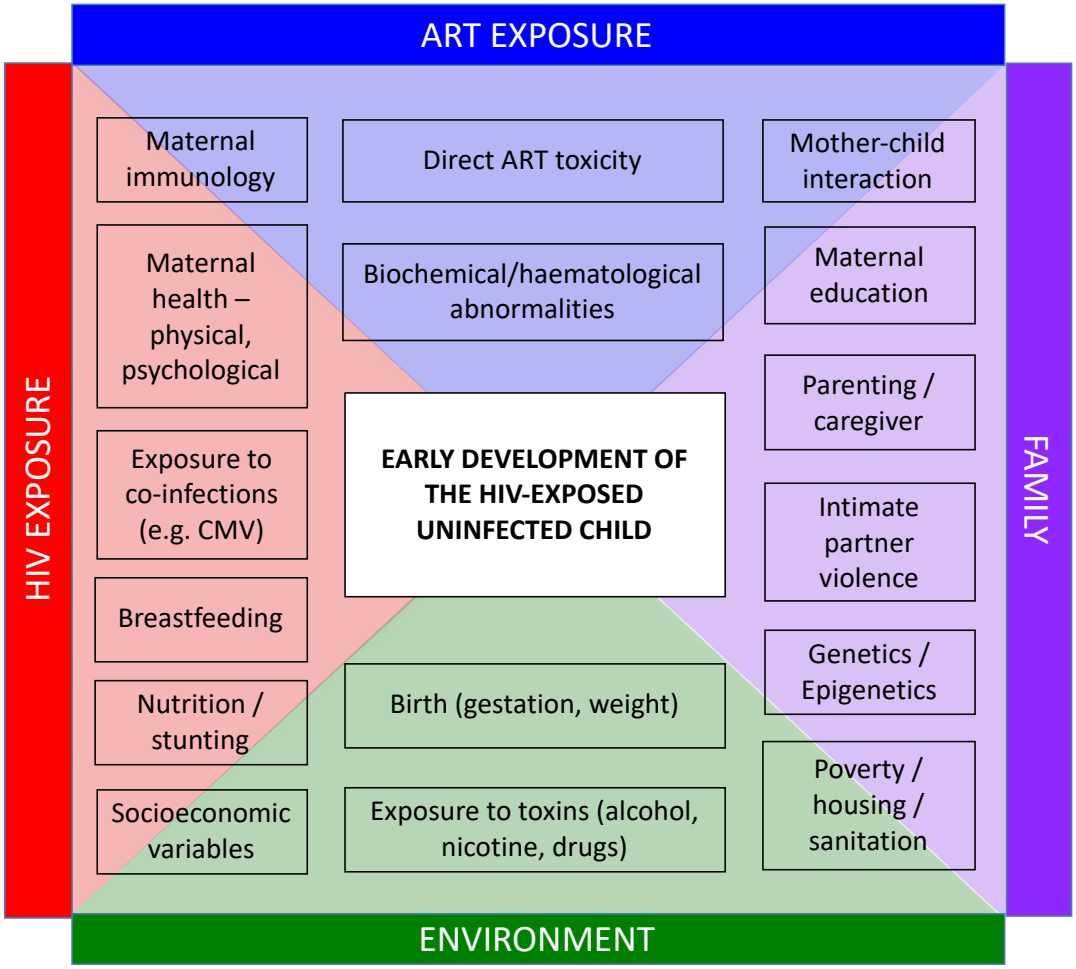

Figure 1.2: Factors influencing the early development of children who are HEU. Figure taken from the book chapter: Wedderburn CJ, Yeung S, Donald KA. Neurodevelopment of children who are HIV-exposed uninfected.

1.3.5 Multifactorial causal pathway

Overall, a multifactorial causal pathway shapes a child's neurodevelopmental trajectory and the accumulation of risk factors puts children at higher risk for neurodevelopmental problems. Both direct effects of HIV and ART exposure on the developing nervous system, and indirect effects associated with being born into a family affected by HIV, may be hypothesised to impact the neurodevelopment of HEU children.[44] Further work is needed to understand the pathways underlying any effects of HIV exposure on neurodevelopment.[44]

1.4 Brain development *in utero* and early childhood

A child's neurodevelopmental trajectory is defined by the development of the nervous system and the neural networks which underly cognition, language, and motor function.[90] Gestation and the early years of life ('the first thousand days') represent the time of most substantial brain development,[91] where critical neural pathway expansion and network maturation occur.[92] This steep anatomical and functional brain trajectory lays the foundations for adult structural and functional brain organisation.[93,94]

Neural development begins 2-3 weeks after conception with neurulation, followed by neuron and glial migration;[95] by mid-pregnancy, most of the billions of neurons have formed. Synaptogenesis then starts, and during the 3rd trimester over 40,000 synapses are created every second.[96] At this time myelination and synaptic pruning begin and continue through early childhood. In the first few years, brain growth is rapid. The brain doubles in size by year one; by age two years the brain is ~80% of an adult volume.[91,97] Longitudinal studies have demonstrated that grey matter drives this early volumetric growth, and increases by over 100% in year one, followed by 10-20% in year two.[97,98] In contrast, white matter increases more slowly and steadily with age.[97-99] There is heterogeneity across the brain with respect to subcortical and cortical morphometric phenotypes, including volume, cortical surface area and thickness, that have different trajectories of growth across regions.[93,94]

The rapid growth and dynamic maturation make the early years a profoundly sensitive and critical period of brain development.[100] Throughout this time, environmental inputs are important to shape brain development, but the complex processes of brain development are also vulnerable to external risks,[54,97,101] which may have a lifelong impact.[54-56] Sensitive periods are defined as times where the brain is particularly responsive to environmental factors, representing periods of heightened vulnerability, but also opportunity, given the plasticity.[102,103] Critical periods are defined as times where a given experience may cause permanent change.[104] As grey matter drives volumetric growth, it is particularly important to investigate factors affecting grey matter maturation during the first years.[97]

Early brain growth is important for later academic outcomes, shaping education performance, employment and mental health.[105] While it is recognised that brain structure underlies behaviour and function, the exact relationships remain to be determined, particularly alongside the rapid brain maturation through childhood. Understanding whether early HIV exposure affects the developing child brain is a key research priority and may help explain adverse neurocognitive outcomes reported in this population.

1.5 Neuroimaging

1.5.1 Neuroimaging techniques

Neuroimaging describes the use of imaging techniques to view the structure and function of the brain *in vivo*. Various imaging modalities have been developed that can directly or indirectly image the brain. These include computed tomography (CT) which uses X-rays; optical imaging which uses infrared light; and magnetic resonance imaging (MRI) which uses magnetic fields and radiofrequency signals to form an image of the brain by leveraging the magnetic properties of different tissues.

MRI is non-invasive and has no radiation exposure or associated specific risks, providing a safe method to assess brain structure and functional connectivity in childhood. There are multiple different MRI modalities that may assess brain architecture, metabolites, and circuitry including: (i) structural MRI, which measures proton signals to map brain structure; (ii) diffusion tensor imaging (DTI) which measures brain organisation and microstructure;[106-108] (ii) magnetic resonance spectroscopy (MRS), which determines levels of metabolites in the brain; and (iii) functional MRI (fMRI) which measures changes in haemoglobin oxygenation levels to detect regional blood flow and therefore neural activity during an active or resting state. While all of these help understand different aspects of brain physiology as well as pathophysiology, across all modalities structural MRI provides a foundational understanding of the neuroanatomy and complements functional neurodevelopmental measures. Structural MRI techniques, including T1- and T2-weighted scans, are capable of mapping and quantifying metrics of brain architecture *in vivo*, including the volume of grey and white matter across the cortical and subcortical regions, as well as the components, cortical surface area and cortical thickness.

Structural MRI technology allows investigation and quantification of neural pathways that underlie neurocognitive and behavioural processes.[109] By advancing our knowledge of neural systems and neurobiology, MRI may be used as a tool to understand patterns of typical early brain development as well as an insight into the mechanisms behind early developmental delay and the origins of later health and disorders.[95,109] Prior neuroimaging studies have shown that environmental factors influence brain development.[56] In particular, grey matter may be affected by *in utero* factors including maternal substance use[110] and child HIV infection.[111,112] Given this precedent, neuroimaging may be used as a tool to understand the effects of HIV exposure on early brain development and delineate pathways underlying any neurodevelopmental deficits that may help to inform strategies to optimise outcomes. However, there is a general scarcity of neuroimaging studies of children from LMICs despite children in SSA having the highest

risk of developmental delay globally. Further, although there are several studies of brain development of young infants and school-aged children in HICs, MRI studies of preschool children between the ages of 1-3 years are lacking.

1.5.2 Challenges of neuroimaging young children

Neuroimaging young children presents multiple challenges to acquisition, processing, and interpretation given the developing neuroanatomy.[113] Firstly, in terms of acquisition, MRIs are particularly difficult to conduct in young children given the noise, duration, and their limited ability to remain still, coupled with the MRI extreme sensitivity to motion.[113] As a result, scans are often performed with sedation in clinical settings. However, there are ethical and risk implications for giving sedation or anaesthesia in a research setting.[114] Performing MRIs during natural sleep is an alternative option, but this is more difficult to initiate and maintain in a noisy scanner and so few MRI studies have investigated the early years of life. Secondly, unique technical and processing challenges exist for imaging young children due to the rapid brain growth and maturation that takes place in the brain during the early years.[54,91,113,115,116] The tissue characteristics of the neonatal brain differ from the adult brain due to ongoing maturation processes that affect T1- and T2-weighted MRI contrasts.[117] The infantile pattern of brain maturation from 0-6 months shows a reversal from the adult grey and white matter contrast, and tissue differentiation is less clear due to the high proportion of unmyelinated white matter.[113] This is combined with the vast increase in brain volume, doubling by one year, that impacts scan parameters and analyses.[91] Thirdly, financial and logistical challenges prohibit MRI scans in LMICs where there is limited access to scanners and participants often have to travel far distances to access MRI machines.[118] This has meant that neuroimaging research has been limited in countries with the highest burden of HIV. In summary, the literature points to a need for MRI studies of young children in LMICs with careful consideration of the logistical, technical, and processing elements involved.

1.5.3 Neuroimaging and HIV exposure

At the start of this thesis, only a small number of studies had used neuroimaging to investigate brain development of children who are HEU. These are summarised in Appendix III.** In 2019, a systematic review described five clinical and multiple preclinical studies of *in utero* exposure to HIV and ART and cognition.[119] Although grey matter drives early volumetric growth, three of the clinical imaging studies investigated white matter microstructure using DTI, one used MRS to examine metabolites, and the final study was a qualitative report of structural scans. Only one study included neonates, and this reported preliminary imaging data from the

** Appendix III also includes more recent publications that are incorporated into the thesis Discussion.

Drakenstein Child Health Study (DCHS) in South Africa. The study demonstrated a difference in brain white matter microstructure in specific regions between HEU and HU infants, including the middle cerebellar peduncles, and correlations between specific brain regions (uncinate fasciculus) and abnormal neurology, as measured by the Dubowitz neurodevelopmental scores.[63] These findings suggested that HIV-associated neurological changes occur early and may impact behaviour. However, more research is needed to ascertain (1) whether grey matter is affected and, (2) whether early changes are transient, reflecting the altered *in utero* environment, or if they persist into later childhood.

Other studies examined older children. Reports from the Children with HIV Early antiRetroviral (CHER) cohort in South Africa showed differences in white matter microstructure and neurometabolites by HIV exposure status in children aged seven and nine years respectively.[120,121] This included differences between HEU and HU children in fractional anisotropy in the corona radiata and corticospinal tract, and lower neurometabolites in the basal ganglia in HEU children. Another study from Thailand examined microstructure in 10-year-old children who are HEU, focusing on the corpus callosum, and found no differences compared to HU children.[122] This study also included a comparison of brain structure using tensor-based morphometry with no differences reaching their defined statistical significance level. However, targeted analyses were not conducted, the sample size was small, and approximately half of the sample was not exposed to ART.[122] Subtle neurocognitive deficits in HEU children were reported in the larger group of children from the same cohort that were not seen in the smaller imaging sample, potentially indicating a lack of power in the imaging analysis.[46]

Only one study from France in 2005 focused on structural imaging in young children. In this study, scans of HEU children aged 10-44 months presenting to hospital with neurological symptoms were subject to expert clinical evaluation (i.e. non-volumetric focus) and examined for qualitative changes.[123] The findings reported 50% of scans showed abnormalities. However, this study had major limitations to generalisability as there was no comparison group (clinical cohort, not a birth cohort), the sample was selected for neurological symptoms with selection bias, brain images were obtained retrospectively without standardized acquisition parameters, and children were predominantly exposed to zidovudine. Overall, there is a scarcity of neuroimaging studies of children who are HEU, in particular, quantitative structural MRI studies of preschool children.

1.6 HIV exposure and neurodevelopment: Evidence gaps

In summary, the evidence for any effect of HIV exposure on child neurodevelopment is inconclusive.[124,52] There is a suggestion that children who are HEU may have poorer neurodevelopmental outcomes than HU children in certain settings, however, more high quality studies are urgently needed to understand this population and any underlying mechanisms, particularly using neuroimaging.[43,44] Over the past decades the population of HEU children has grown considerably alongside changing international guidelines, and there is a lack of data from the current ART era.[42] Advances in neuroimaging provide novel approaches to investigate mechanisms that underlie neurodevelopment, but few studies have utilised this technology to examine HEU children. To date no published research has examined the effects of antenatal HIV exposure on grey matter maturation in the early years of life. The first three years represent a dynamic period of structural brain development that lay the architectural foundation for behaviour and function.[54] Even a small impact on early cognitive and behavioural skills may substantially affect schooling, job opportunities and long-term health outcomes. Therefore, studying neurodevelopmental outcomes alongside early brain subcortical and cortical structure during this time will provide a better understanding of the neuroanatomical underpinnings of neurocognitive function,[125,126] and factors affecting grey matter maturation during this period.[97] This thesis seeks to address critical questions regarding the relationship between HIV exposure, neurodevelopmental outcomes, and brain structure, as well as the role of neuroimaging as a tool to investigate brain development.

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2

Chapter 2: Study Rationale, Hypothesis, Aim, and Objectives

Study Rationale, Hypothesis, Aim, and Objectives

Summary

This chapter contains linking material summarising the overarching rationale for the research and location of the study followed by the hypothesis, aim, and objectives of this study. A figure illustrating the study and outcome measures is given.

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2.1 Rationale: Research aim

The proposed research addressed the need for prospective studies to investigate the effects of HIV exposure on child brain development (structural and functional) in the first years of life in the ART era. It also examined the impact of ART exposure on children and the role of MRI in understanding neurodevelopment of children who are HEU. This has substantial public health relevance given the growing population of children who are HEU, the majority of whom live in SSA. Early child development has a lasting impact on physical and psychological health outcomes affecting schooling, health, and social potential.[1] Therefore, even minor neurodevelopmental impairment may have critical implications, both from an individual and a societal perspective, particularly in locations where children who are HEU represent a substantial proportion of each new generation.[2] Studying the effects of HIV exposure on neurodevelopment now becomes a key priority to achieve the Sustainable Development Goals.[3,4] This study may inform healthcare workers and policy-makers regarding the potential future healthcare needs of this growing vulnerable population and intervention strategies to improve neurodevelopmental outcomes of children who are HEU.

2.2 Rationale: Study location

South Africa has successfully implemented vertical transmission prevention programmes, however, antenatal HIV prevalence remains high (~21-25%) and the country has the highest burden of HIV in the world making it a relevant location.[5,6] The University of Cape Town has the infrastructure and technology to support state-of-the art neuroimaging and the Cape Universities Body Imaging Centre (CUBIC) hosts the first dedicated research MRI scanner of its kind in Africa. A large observational population-based birth cohort study is currently ongoing in the Western Cape of South Africa investigating early-life child health outcomes: the Drakenstein Child Health study (DCHS).[7] The DCHS has a well-characterised cohort of over 1000 mother-child pairs with comprehensive sociodemographic, HIV, and ART data and ongoing follow up. Nesting the study within the DCHS allowed a longitudinal study design; an appropriate HU comparator group from the same community to reduce confounding; and information on biological, social and environmental factors to address the research gap for the role of covarying factors. Preliminary neonatal data from the DCHS study has demonstrated the potential for neuroimaging in this setting.[8] This provided a unique opportunity to undertake a prospective nested neuroimaging study to investigate the impact of HIV exposure on child neurodevelopment and the relationship with neuroanatomy. The sociodemographic context of the DCHS is typical of many SSA countries and therefore data emerging from this study may have generalisability and contribute to our understanding of the health outcomes of HEU children globally.

2.3 Research questions:

(i) How does HIV exposure affect child neurodevelopment and brain structure?

(ii) How does neurodevelopment and brain structure change over the first three years in children who are HEU?

(iii) Do neuroimaging findings correlate with neurodevelopmental outcomes in children who are HEU?

To address these research questions, this thesis includes a prospective study nested in the DCHS comparing neuroimaging and neurodevelopmental assessment outcomes of HEU children with a group of HU children (Figure 2.1), a conceptual framework, and a systematic review and meta-analysis.

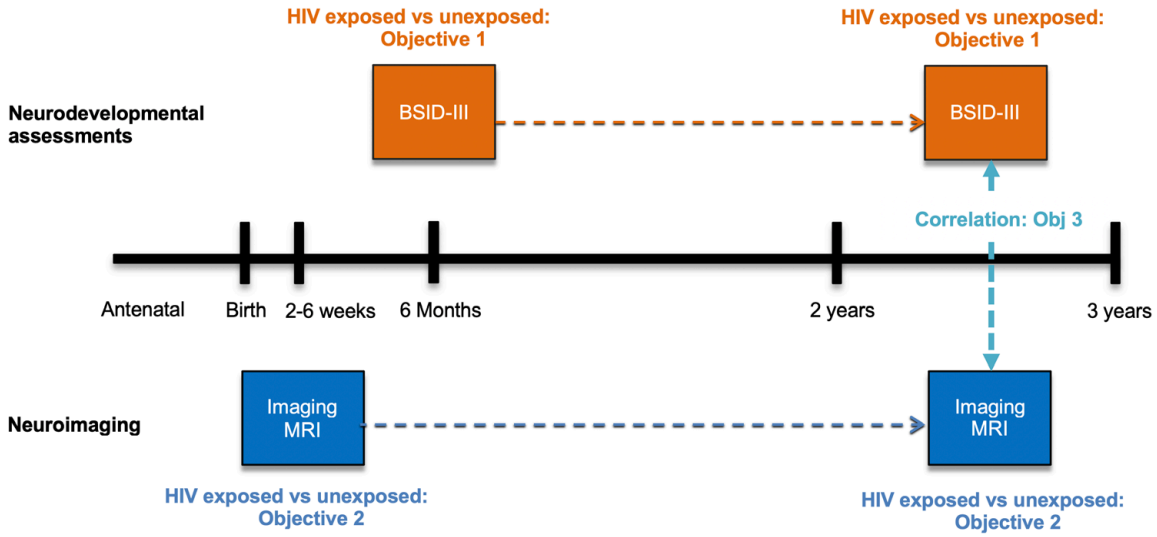

Figure 2.1: Timeline of study outcome measures
Legend: Illustration of the age at which the outcome measures were performed to address the objectives (see Section 2.6 below). BSID-III: Bayley Scales of Infant and Toddler Development, 3rd Edition; MRI: Magnetic Resonance Imaging.

2.4 Hypothesis

HIV exposure adversely affects the brain structure and function of children who are HEU compared to HU children from the same environment.

2.5 Aim

The overarching aim of this thesis was to examine the effects of exposure to maternal HIV infection on the neurodevelopment and neuroanatomy of uninfected children born to mothers living with HIV in the ART era.

2.6 Specific objectives

Primary objectives:

1) To compare neurodevelopmental outcomes (cognition, language, motor function) between children who are HEU and HU in infancy (6 months) and early childhood (2-3 years).

2) To investigate group differences in brain structure in children who are HEU compared to HU using magnetic resonance imaging in early infancy (2-6 weeks) and early childhood (2-3 years).

3) To determine whether there is an association between structural neuroimaging findings and neurodevelopmental outcomes in children who are HEU.

Secondary objectives included examining the associations between maternal HIV disease severity (as measured by CD4 cell count and viral load) and ART with child neurodevelopment.

2.7 References

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3

Chapter 3: Study Protocol and Methods

Study Protocol and Methods

Summary

This thesis provides a comprehensive assessment of brain structure and function of children who are HEU using well-validated measures of neurodevelopment and state-of-the art neuroimaging techniques. This chapter includes details of the study protocol, data collection, and methodology of the empirical study. The research is nested in the Drakenstein Child Health Study and I describe the study setting and enrolment of both the parent and sub-study. While the specific methodologies and statistical analyses are described in detail in the respective research papers, in this chapter I give the broad overview. At the end of the chapter, I describe the ethical considerations and my role in the methods and data collection.

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3.1 Study design

To address the study aim, a prospective observational study examining the clinical neurodevelopment and brain structure of children who are HEU nested within the Drakenstein Child Health study (DCHS) was conducted. High resolution structural MRI and comprehensive neurodevelopmental assessments were undertaken in a group of HEU children aged 2-3 years and compared with HU children from the same community to address objectives 1, 2, and 3 (Chapter 2.6). Data that had already been collected were available for secondary data analysis to provide an assessment of brain structure and function at earlier timepoints.

The DCHS is a large prospective population-based birth cohort investigating the early-life determinants of child health and development.[1,2] The overview methods of the DCHS have been described previously,[1,3] along with the psychosocial components.[2] Given the well-characterised population with comprehensive sociodemographic, environmental, and HIV data, the study provided a unique opportunity to undertake a prospective study of the impact of HIV exposure on child neurodevelopment.

3.2 Study setting

The DCHS is set in the Drakenstein region of Paarl, a deprived peri-urban area 60 km outside Cape Town, Western Cape, South Africa (Figure 3.1). This sub-district was specifically chosen for its established healthcare infrastructure, stable population of approximately 200,000, accessibility to Cape Town, high burden of infectious diseases and low socioeconomic status similar to other LMICs (Figure 3.2). There is a good primary health care infrastructure with 23 primary healthcare facilities and one referral hospital. An estimated 90% of women use public healthcare maternity and child services. The study sites comprise public health care facilities: Paarl Hospital and two primary care clinics, Mbekweni (serving a predominantly black African community) and TC Newman (serving a predominantly mixed ancestry community) clinics, which provide free healthcare.

Figure 3.1: Map of South Africa showing the relative position of the study site in Paarl (red dot). [From Google maps]

3.3 Study population

3.3.1 Population of parent (DCHS) study

The DCHS enrolled mothers at 20-28 weeks' gestation over a three year period, between March 2012 and March 2015. Pregnant women who were attending the specified clinics for antenatal appointments at 20-28 weeks' gestation were screened and invited to enter the study if they fulfilled the inclusion and exclusion criteria (Table 3.1). Enrolment was unfiltered, without any selection for sociodemographic risk factors, and every mother who presented to the clinic in the correct gestational window was offered to be part of the study. Women completed written informed consent forms at enrolment, which are renewed annually as follow up of mothers and children is ongoing. Regular follow up visits coincided with routine postnatal visits initially, followed by every 6 months at the primary health care clinics and annual study visits at Paarl Hospital.

In total 1225 mothers were enrolled, and 1137 mothers gave birth to 1143 live births (including four sets of twins and 1 set of triplets) (Figure 3.3). Antenatal maternal HIV prevalence was 21%, but only two infants were HIV-infected due to the strong PMTCT programmes.[3] Cohort recruitment was completed in 2015 and longitudinal follow up is ongoing. There is strong cohort retention to date with community buy-in and less than 10% loss to follow-up a year; 1002 children remained in the cohort at two years.

Figure 3.2: Photos of study site. Source: DCHS stock pictures

3.3.2 Population of nested neuroimaging study

A neuroimaging sub-study was embedded in the larger Drakenstein Child Health Study. After birth, a sub-group of infants were invited for neuroimaging if they fulfilled sub-study inclusion and exclusion criteria (see Table 3.1; hereafter referred to as the "neuroimaging sub-group"). Infants were included if they were aged 2-6 weeks and excluded if they had comorbidities known to be associated with neurodevelopmental impairment (listed in Table 3.1). Mothers in the cohort who had given birth 2-3 weeks earlier were approached and asked if their newborns could be included in the neurodevelopmental sub-study.[4] In total, 236 infants were in the neuroimaging sub-group (20.6% of the full cohort) and attended for scans between 2012 and 2015.

On reaching two years of age, children from the neuroimaging sub-study who remained active in the cohort and stayed in the study area were invited for repeat imaging at this time-point. Additional children from the parent DCHS not imaged at birth were included to reach sample size given loss to follow up, selecting for risk factors (maternal HIV) to ensure a representative sample of the high-risk population, along with a randomly selected comparison group (Table 3.1). A total of 239 children (23.9% of the cohort at two years) were recruited for neuroimaging at 2-3 years with a pilot phase from July to December 2015, and the main study from January 2016 to September 2018. This neuroimaging was developed and conducted as part of this PhD, and the neonatal scans were available for processing and analysis.

Table 3.1: Inclusion and exclusion criteria of the DCHS and the neuroimaging sub-study

	Inclusion Criteria	**Exclusion criteria:**
Parent (DCHS) study (*n=1143)*	Antenatal Clinic attendees ≥18 years Pregnant and 20-28 weeks' gestation Receiving antenatal care at a participating study site	Living outside of the region or planning to move out in the next year
Neuroimaging sub-group of the parent study *(n=236 at 2-6 weeks; n=239 at 2-3 years) 20-25% of DCHS cohort*	***Neonatal group:*** Age 2-6 weeks ***2-3 years:*** Age 2-3 years Neonatal imaging prioritised Additional selection for HIV exposure. HU children were identified in a 1:1 ratio by age, sex and clinic.	(i) Medical comorbidity including a genetic syndrome, neurological disorder, or congenital abnormality (ii) History of prematurity (gestation <36 weeks) (iii) Low Apgar score (<7 at 5 minutes) (iv) Neonatal ICU admission (v) Maternal use of illicit drugs during pregnancy (e.g., cocaine, methamphetamines, opioids) (vi) MRI contraindications (e.g., ferromagnetic implants) (vii) child HIV infection

Written informed consent was obtained from mothers at enrolment and mothers resubmit their consent annually. Additional written informed consent was given by the parent/guardian at the neuroimaging visits.

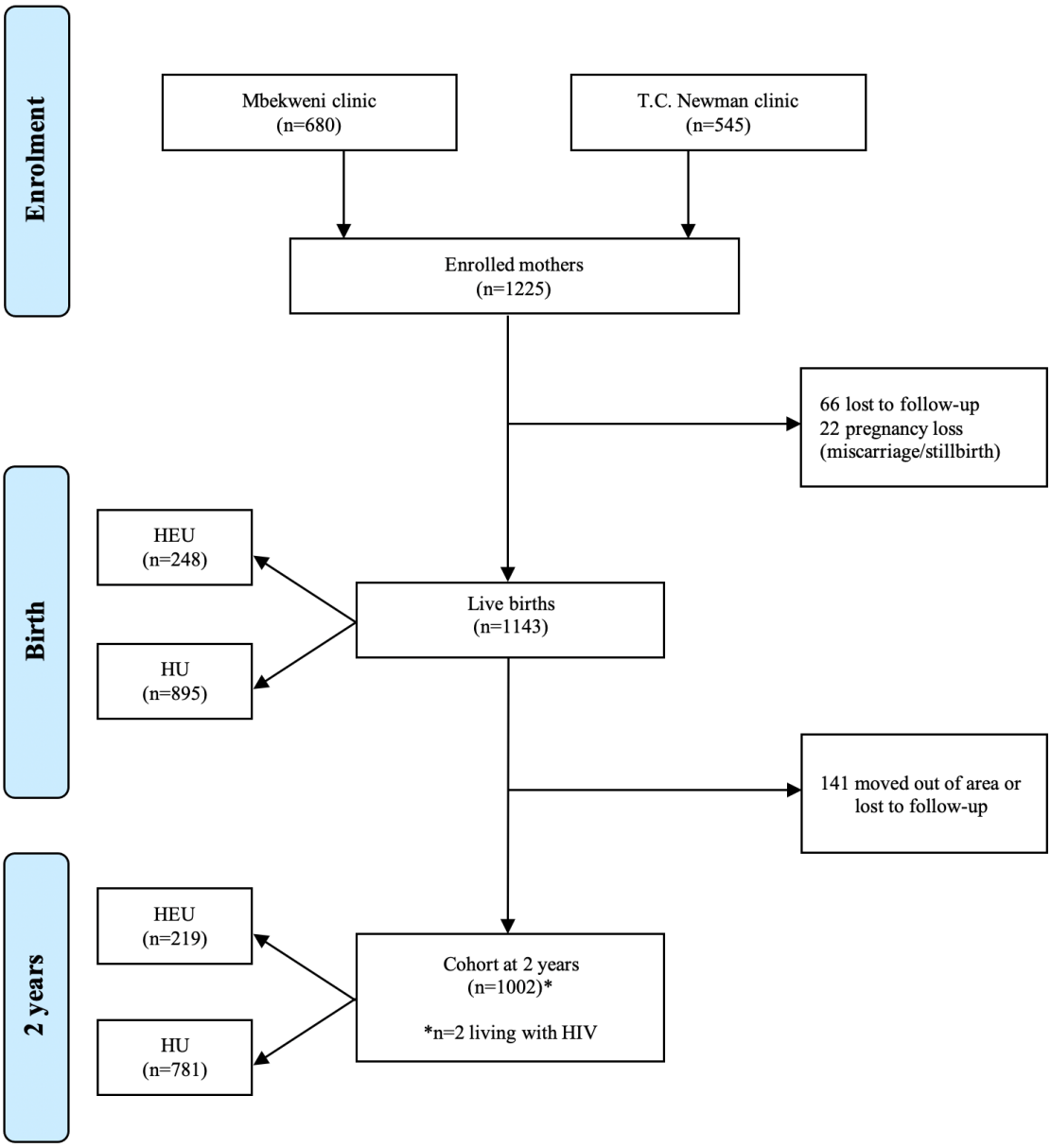

Figure 3.3: Drakenstein Child Health Study Flow Diagram

3.4 Data collection

The following sections describe the instruments and data collection techniques performed to address the main objectives of the study: neurodevelopment and neuroimaging.

3.4.1 HIV/ART data

Mothers and children were tested for HIV as per South African national guidelines. All pregnant women and children living with HIV were initiated on ART as part of the South African PMTCT programme.

HIV status was established at enrolment. As per South African PMTCT guidelines, HIV testing of pregnant women was performed routinely by clinical staff, unless they were already known to have HIV infection. HIV-negative women were retested during pregnancy and breastfeeding every 3 months. Additional maternal interviews and HIV status reviews were conducted by DCHS study staff at birth, 6 weeks, 14 weeks, 6 months, and 6 monthly thereafter. HIV-exposed children were tested at 6-10 weeks of age using a PCR test, 9 months and 18 months using a rapid test. At 18 months, all HIV-exposed children remaining in the cohort study area were confirmed to be HIV negative through either a negative 18 month HIV test or negative test post-cessation of breastfeeding, or were formula-fed with a negative HIV test after 6 weeks. Positive diagnoses were confirmed using a second test. At 18 months post-partum only one mother had seroconverted and there were two HIV infections in children in total. Further details can be found in *Pellowski J, et al,* 2019.[5]

All mothers with HIV were enrolled onto the national PMTCT programme and initiated on ART. All children with HIV were referred to the appropriate clinic for treatment. The first line ART regimen in South Africa for the majority of mothers at enrolment was efavirenz, tenofovir, and emtricitabine as a fixed dose combination with nevirapine or nevirapine plus zidovudine given as neonatal prophylaxis. However, as the study recruitment spanned 2012-2015, some women gave birth under the Option A guidelines recommending either zidovudine or ART to pregnant women with HIV depending on their CD4 cell count at diagnosis, while others gave birth under Option B/B+ which was introduced in 2013 recommending triple ART to all pregnant women regardless of their health status. Consequently, there is some heterogeneity in the antiretroviral combinations and maternal immune status across the cohort which is representative of clinical populations in other LMIC settings across SSA. Clinical measures of maternal CD4 cell count and plasma viral load measurements, and treatment history were collected from the National Health Laboratory Service and through clinic and folder reviewers. Children who are HEU were defined as those children born to mothers with HIV but who remain HIV-free. HU children were born to mothers who tested negative for HIV.

3.4.2 Neurodevelopmental assessment

Neurodevelopment was assessed in a sub-group of children at 6 months, and in the full cohort of children at two years. The subgroup of children was randomly selected from the original cohort as a sampling frame for developmental assessment at 6 months of age, blinded to HIV status. If a mother-child pair was unavailable, another child aged 6 months was invited to attend from those with study follow up visits that week, based on child age at the time. All available children were assessed at two years. Mothers who were unavailable at initial contact were tried again up to three times. Children who were unwell on the day were rebooked where possible.

Children were assessed using the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III), an international gold-standard measure of development validated for use in South Africa (Table 3.2).[6,7] The BSID-III is a comprehensive tool to assess child development from 0-42 months in 30-90 minutes, and is an extensively employed measure of early neurodevelopment worldwide. It has five scales: (1) Cognitive; (2) Language (separated into receptive and expressive language components); (3) Motor (with fine and gross motor components); (4) Social-emotional; and (5) Adaptive behaviour. The first three scales were assessed by direct observation by a trained assessor; the latter two subscales were assessed indirectly by caregiver-completed questionnaires. Earlier versions of the tool have been used to assess children who are HEU in the Democratic Republic of Congo[8] and Tanzania.[9] The tool has been validated previously in South Africa to assess children with neurodevelopment disabilities[10] and very low birth weight infants,[7] and was found to be a suitable tool for use. A high inter-observer concordance was found between testers (98%).

Table 3.2: Summary of the Bayley Scales of Infant and Toddler Development (BSID-III)

Summary	Administration & Scoring
International Gold-standard measure of development, sensitive to subtle developmental delay.[6] Designed to assess development in children from 1-42 months of age.[6,11] Validated for a South African population.[7,10] Widely used measure in other cross-cultural contexts. Scales: (1) Cognitive (2) Language (3) Motor (4) Social-emotional (5) Adaptive behaviour.	Administered at 6 months and 2 years of age. The first three scales are assessed by direct observation, and the latter two through a questionnaire completed by the caregiver. Produces norm-referenced scores across subscales. Standardised assessments (physiotherapist, occupational therapist) to ensure concordance. Scored according to manual and software.

The most current version of the tool at the time, the BSID-III, was chosen in this study as an age-appropriate tool for use in a South African population aged 6–24 months, and the directly observed scales are the focus of this thesis. Trained assessors (paediatric physiotherapist and occupational therapist) performed the assessments with language prompts in the child's preferred language (Afrikaans or isiXhosa). The assessors were healthcare professionals who alternated testing between the two clinics (TC Newman and Mbekweni) and assessed equal numbers of children at each site in both age groups, blinded to HIV status. They were supervised by a Paediatric Neurodevelopmental Specialist who oversaw assessments for the duration of the study to ensure compliance with the test manual and standardized data collection. Inter-reliability was assured through training and supervision; assessors were periodically required to complete an assessment under observation to ensure overall accuracy in the assessment process.

External quality monitoring was implemented in accordance with best practice and reports in the literature of human administrative and scoring errors. Systematic quality checking was done regularly to ensure fidelity to the detailed instructions for data scoring and administration. A second level of quality control was performed centrally to check the scoring. Scores were entered into the Bayley's specialised software and checked to ensure quality control. Raw scores, scaled scores (performance related to peers controlling for age and sex) and composite scores (mean and standard deviation) were reported. Scores in the 6-month age group were adjusted for prematurity where relevant per BSID-III manual guidance.

Any significant neurodevelopmental delay was noted by the assessors and children were referred for care, with permission from the parents, via local referral pathways to the appropriate clinical service. Vision and hearing were tested separately, however, deficits that meant assessments could not be completed were noted by assessors at the time.

In addition, and outside the scope of the core thesis, the children in the cohort underwent further cognitive assessment at 3.5 years of age. This was undertaken using a comprehensive and validated tool battery that measures general cognitive function, executive function, memory, language and motor control, the Kaufman Assessment Battery for Children, 2nd Edition (KABC-II). These domains were carefully selected to cover critical areas of development and potential for school achievement. This is a standard neurocognitive test battery suitable for children aged 3.5 years that is reliable and validated sufficiently for the South African context. The outcomes are included in Appendix XI.

3.4.3 Neuroimaging assessment

Multimodal imaging followed a standard local protocol developed for the study. The neonatal imaging took place during natural sleep from 2012 - 2015, prior to this thesis. Full details of the neonatal imaging methods are included in Chapter 8. I undertook the image processing with supervision and conducted the statistical analysis but was not involved in the data collection. The 2-3 year old imaging took place between 2016 - 2018 and I carried out the data collection as part of this thesis. Figure 3.4 shows the study team and full details are included below and in Chapter 7.

Figure 3.4: Study neuroimaging team.

3.4.3.1 Image acquisition

High resolution MRI images of each child were taken using the same 3 Tesla Siemens Allegra MRI scanner (Erlangen, Germany) at the Cape Universities Body Imaging Centre (CUBIC) in South Africa with a 32-channel head coil (Figure 3.5). Due to the challenges of conducting imaging in young children, the scans were performed when the children were asleep to limit motion in order to obtain high quality images. No sedation or anaesthesia was used (see Chapter 7 for further details). The imaging centre is child-friendly and the scans were conducted around the time of their lunchtime nap or later in the day when the children were more likely to sleep at their natural bedtime. Children were brought to the imaging centre mildly sleep-deprived, and given a drink and a meal on arrival and melatonin (a naturally occurring substance) to help initiate sleep as per current local imaging study protocol. The mother was then asked to put the child to sleep in a low-lit room on a bed or on their back with the study team on hand for sleep advice. As soon as the child was in a deep sleep, he/she was placed in the brain scanner and ear protection was fitted. This capitalised on the sleep cycle, where certain states have a higher arousal threshold and lower spontaneous movements. Sleep stage 3 fits these criteria and happens early in the sleep cycle after sleep

initiation in younger age groups. Imaging during natural sleep was also successfully undertaken during the neonatal scanning of 236 infants using a feed and swaddle approach.

A study nurse or doctor was present in the scanner at all times to detect any signs of the child waking up. A slot was booked for a total scan time of an hour with extra time to allow for settling the child into the scanner and in case the child needed resettling during the session; if the child woke up the scan was immediately stopped. The scans were reported by a clinical radiologist and any incidental findings reported on the imaging were referred through the appropriate local clinical pathways and discussed with the parents.

Figure 3.5: CUBIC Imaging Centre, Groote Schuur Hospital, University of Cape Town

3.4.3.2 Imaging protocol

Figure 3.6 details the structural MRI technical protocol, and Figure 3.7 shows a child in the MRI scanner. This is a standardised protocol recommended by neurophysicists and neuroradiologists which balances out time and quality of scans utilising the best techniques available, similar to the imaging that was undertaken in the children at 2-6 weeks of age.

Instrument: Imaging was acquired at the University of Cape Town Cape Universities Body Imaging Centre on a 3 Tesla Siemens Magnetom Skyra, Siemens Germany, Erlangen,70 cm diameter bore, whole body MRI scanner. A 32 channel head coil was used. **Structural sequence:** Sagittal T1-weighted multi-echo magnetisation prepared rapid gradient echo (MEMPRAGE) images were acquired using the parameters: repetition time (TR) = 2530; echo time (TE) = 1.69, 3.54, 5.39, 7.24; inversion time (TI) = 1100; field of view (FOV) = 224 mm; matrix =256 x 256, 176 slices, 1.0 mm thick. flip angle= 7.0°; voxel size= 1.0×1.0×1.0mm

Figure 3.6: T1-weighted structural imaging technical sequences protocol

Other imaging modalities including diffusion tensor imaging, resting state blood-oxygen-level dependent (BOLD) signal sequences and magnetic resonance spectroscopy (MRS) were also captured as part of the original study protocol. The full scan protocol was 58 minutes 38 seconds in duration and sequence details are included in Chapter 7.

Figure 3.7: Child participant after an MRI scan. Photo with parental permission.

3.4.3.3 Image processing

After the image acquisition, structural MR images were processed using pipelines described in the individual papers. Structural image sequences were processed using standard software programmes to extract total and subcortical volumes (2-6 weeks and 2-3 years) and cortical metrics (surface area and thickness) (2-3 years only) as the neuroimaging outcome measures. Brief summaries are given below.

Both T1- and T2-weighted scans were performed. At 2-6 weeks, T2-weighted sequences were processed to investigate brain volumes due to the improved contrast at this age. Neonatal brains have high proportions of unmyelinated white matter and therefore a higher water content than adult brains, and lower protein and lipid contents. This leads to prolonged relaxation times (T1 and T2) that show a reversal from the adult grey and white matter contrast affecting T1- and T2-weighted MRI contrasts.[12] Due to the infantile pattern of brain maturation from 0-6 months, grey matter and unmyelinated white matter have similar signal intensity on T1-weighted images in young infants, and differentiation is less clear. Therefore, T2-weighted images are more commonly used for the segmentation of grey matter in young infants,[12,13] and preferred as an index of early brain development.[14,15] T2-weighted images

were analysed using Statistical Parametric Mapping 2 (SPM2) in Matlab (MathWorks, Natick, MA). Segmentation and non-linear normalisation were performed.

At age 2-3 years, T1–weighted MP-RAGE (Magnetization Prepared Rapid Acquisition Gradient Echo) sequences were processed to examine cortical and subcortical regional volumes in children. T1-weighted imaging best delineates brain anatomy at this age with segmentation into white matter, grey matter and cerebrospinal fluid. Tissue volumes were calculated globally and for regions of interest (ROIs). ROIs allow study of specific anatomical areas using atlas-based techniques.

For the 2-3 year olds, scans were processed through the FreeSurfer programme using the automated recon-all command at the Centre for High Performance Computing, Cape Town. FreeSurfer version 6.0 (http://www.freesurfer.net) was used for automated cortical reconstruction and to measure regional and total brain volumes, cortical thickness and cortical surface area.[16,17] Similar to the processing for the neonatal scans, the pipeline includes motion correction, skull stripping, volumetric labelling, normalisation and segmentation into white and grey matter, with additional surface atlas registration and reconstruction for cortical surface area and thickness, parcellation and labelling (Figure 3.8).

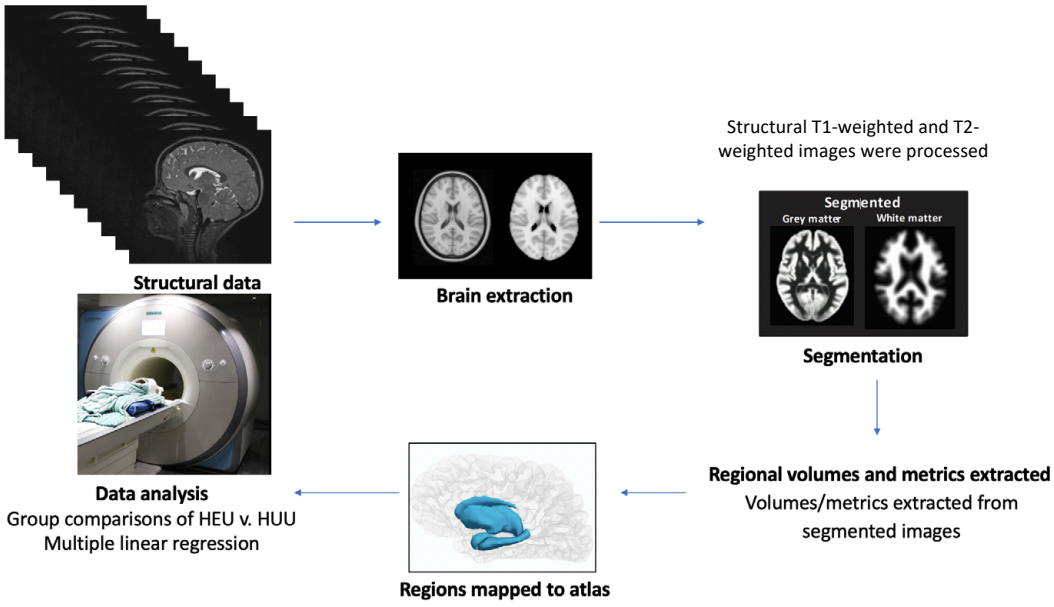

Figure 3.8: Overview of the Image Processing Pipeline

Quality control procedures were implemented. Structural scans were manually assessed, blinded to HIV exposure status, for image quality. Raw data were visually checked to ensure images had no distortions. Processed outputs were visually inspected for errors in segmentation of cortical and subcortical structures. Subjects were further investigated if their mean values on these measures were classified as extreme outliers.[18]

3.4.4 Sociodemographic data

Sociodemographic data were collected through the DCHS at enrolment, birth and at subsequent visits, relating to infectious, nutritional, environmental, socioeconomic, and psychosocial variables of the children and parents through a mixture of self-report and researcher-administered standard measures (Table 3.3).

Table 3.3: Variables relating to Maternal and Child Health

Category	Components	Measures
Maternal Health & Socioeconomic status	Maternal health	Standardised questionnaire at routine DCHS visits
	Maternal education	Sociodemographic questionnaire – adapted from items used in the South African Stress and Health Study (SASH)[19]
	Employment	
	Household income	Classified monthly in rand
	Relationship status	Married/cohabiting or single
Disease severity / Immunological	Maternal CD4 cell count, viral load, ART	Routine CD4 and viral load measures for mothers living with HIV coupled with ART questions
Environmental	Maternal alcohol intake	Alcohol, Smoking and Substance Involvement Screening Test (ASSIST)[20] validated for local pregnant populations; Retrospective measure of alcohol use during pregnancy devised for this study done at birth and at 2 years. Composite variable created.
	Substance use	Rapid urine dipstick testing
	Smoking	Questionnaire pre- and post-natally; Urine cotinine as a biomarker of tobacco smoke exposure
Psychosocial	Maternal Depression	Beck depression inventory (BDI-II)[21] SRQ-20 self-report measure of psychological symptoms[22] Edinburgh Postnatal Depression Rating Scale (EPDS)[23,24]
Infectious	Child illness Maternal illness	Children receive routine investigations as part of the DCHS. Hospital admissions and illness episodes recorded
Nutritional	Infant assessment Infant feeding Anthropometry Diet history; food security	Nutritional history including birth weight and length, gestational age, infant feeding practices. Child weight, height and head circumference were taken at imaging. Growth measures, dietary history and food security assessments (adapted from Bickel G et al 2000[25]) are collected during routine DCHS visits.
Child	Birth history	Delivery mode, gestation, birth weight, APGAR score, resuscitation

Trained interpreters were used where necessary and measures were translated into isiXhosa and Afrikaans using standard forward and backwards translation. Hospital and clinic records were screened for antenatal and birth information, including anthropometry. Anthropometric measurements (weight, height, head circumference) were also recorded at the imaging sessions.

3.5 Data analysis

All analyses were completed using STATA version 14 or 15 and are documented in the individual data papers. The general approach included assessing data for normality and parametric or non-parametric tests were conducted accordingly. Sociodemographic data were compared between HEU and HU groups. To address objective one, comparisons were made between HEU and HU children for neurodevelopmental outcome measures, and to address objective two, for neuroimaging measures (Table 3.4). Analyses of differences between groups at each time point were made. For the neurodevelopmental analyses, group-wise comparisons were made across the whole DCHS cohort.

Data were analysed unadjusted and then adjusted for potential confounders selected using a directed acyclic graph. Sub-analyses were carried out to examine the association between maternal severity of disease (as measured by CD4 cell count and viral load), specific ART regimens and initiation timing. To address objective 3, analyses were undertaken to explore the relationship between imaging outcome measures, exposure to HIV, and clinical neurodevelopmental outcome measurements.

Table 3.4: Statistical Analysis Plan

OUTCOME MEASURE	AGE AT MEASUREMENT AND SCORING

MRI (structure)
• Total grey matter volume
• Total subcortical volume
• Individual subcortical region volumes
• Cortical regions of interest:
  ○ surface area and thickness
  ○ (2-3 years only)
	Continuous outcome measures
	
	Age 2-6 weeks and 2-3 years

Neurodevelopment (BSID-III) • Cognition • Receptive language • Expressive language • Fine motor • Gross motor	Standardised scores, continuous measures and dichotomised into delay Age 6 months and 2 years

3.6 Sample size

A power analysis for the 2-3 year old neuroimaging was based on preliminary neonatal imaging data from the DCHS which had found a difference in white matter microstructure between HEU and HU neonates.[26] The power analysis indicated a sample size of 80 (n=40 in each group) would give more than 80% power to detect a standardised effect size of 0.66 and above in the DTI metric for white matter integrity, fractional anisotropy, between groups at a

significance of p=0.05 (Table 3.5). DTI was the most difficult neuroimaging outcome measurement to obtain and was therefore chosen to estimate the minimum sample size.

Table 3.5: Power calculation

SD 0.04			SD 0.03		
Difference between the mean FA of HIV-exposed children compared to HIV-unexposed	Effect Size	Power	Difference between the mean FA of HIV-exposed children compared to HIV-unexposed	Effect Size	Power
0.05	1.25	1.0	0.05	1.67	1.0
0.04	1	0.993	0.04	1.33	1.0
0.03	0.75	0.91	0.03	1	0.99
0.02	0.5	0.60	0.02	0.66	0.84

Footnote: SD: Standard deviation. Power to detect a difference in the fractional anisotropy of a specific region between HIV-exposed and unexposed groups using a sample size of 80 (40 children in each group) across a range of effect sizes. The mean is a measurement of fractional anisotropy, a measure of white matter structure and integrity, which ranges from 0-1 and is estimated from the neonatal data with allowance for brain growth. The values and SD are taken from the neonatal measurements, with assumption that FA values get larger and SD gets smaller with age, two different SD are presented[27]. Given these data, a sample size of 80 will give over 80% power to detect an effect size of >0.66. The power remains the same when detecting a difference in the mean change in the HIV-exposed group compared to the mean change in the unexposed group over time (longitudinal analysis).

3.7 Ethical approvals

The research for this thesis was conducted as a nested sub-study within the DCHS. This nested study has specific approval from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC 044/2017) and the London School of Hygiene & Tropical Medicine Observational Ethics Committee (11903). The DCHS has ethical approval from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC 401/2009), Stellenbosch University (N12/02/0002) and the Western Cape Department of Health Provincial Research Committee (2011RP45). The longitudinal neuroimaging has additional approval (HREC 525/2012).

3.8 Ethical considerations

All study procedures and outcome measures detailed in this nested study were covered by ethical approvals. The neurodevelopmental and neurocognitive assessments as well as the collection of clinical data were included in the approved DCHS protocol. The magnetic resonance imaging was included in the approved neuroimaging protocols and does not involve radiation, contrast, or other harm to children. Children with identified neurodevelopmental impairment were referred to appropriate healthcare professionals and any incidental neuroimaging findings were referred for management through appropriate clinical pathways. Management and communication followed local procedures.

Written informed consent was taken from all mothers at enrolment by trained study staff in the participants' home language as part of the DCHS and this is renewed annually. Additional written informed consent was obtained before each imaging session from the parent/legal guardian accompanying the child. Prior to the imaging sessions, a trained member of the study team explained the study procedures and the purpose of the study in a quiet consultation room in CUBIC with the aid of a mock scanner as necessary. Adequate time was allowed for questions in the parent/legal caregiver's preferred language with the aid of a translator as necessary. Any concerns during the imaging session were addressed in a timely manner and the parent/child were able to terminate the session at any point and to withdraw from the study. Participants' parents/caregivers had their travel costs reimbursed for any visits that fell outside of well child clinic visits. For imaging sessions that took place in the evenings, transport was provided and parents/caregivers had their time and meal costs reimbursed appropriate to the locality.

All participant data were collected and managed in an ethical manner in accordance with research ethics committee stipulations, maintaining privacy and data confidentiality at all times and adhering to Good Clinical Practice principles. Stringent processes for management of data and ensuring confidentiality were followed and are detailed in the data management plan in Appendix IV. All data were anonymised from point of collection and linked by unique participant identifiers. Security processes are in place with access permissions to protect the data. Clear guidance was provided to ensure the privacy of the participants and communication was aligned with the established DCHS to ensure confidentiality and that participants could communicate with the research team at any point. The protocol complies with the latest version of the Declaration of Helsinki, The Department of Health: Ethics in Health Research: Principles Structures and Processes, and Good Clinical Practice guidance. The study is covered by UCT's no-fault insurance.

3.9 My role and contributions

The DCHS is a population-based birth cohort with contributions from a large number of people, many of whom are included in the list of contributors and co-authors on pages 18-19 and the acknowledgements. My specific roles are outlined below and include conceptualisation of work in this thesis in collaboration with my supervisors and the DCHS investigators. Prior to the work, I conducted a provisional literature search which demonstrated: (i) there was a need for longitudinal studies examining the neurodevelopmental impact of HIV exposure in the current era of lifelong ART which provided the rationale for my study; and (ii), there was scope for an updated systematic review which included more

recent literature and separated ART exposure where possible. I therefore conceived and obtained funding for the study of neurodevelopment of children who are HEU nested within the DCHS. I used a broad range of research methodologies, and I elaborate on the conceptual framework in Chapter 5 and the comprehensive systematic review and meta-analysis in Chapter 6.

Working with the DCHS data team and the study doctor at Paarl Hospital, I oversaw processing and quality control of the cohort maternal and infant HIV/ART and breastfeeding data, and confirming child HIV status, which involved additional data collection and working with multiple data sources. BSID-III assessments were carried out by a paediatric physiotherapist and occupational therapist. Following completion of a BSID course, I led the monitoring, scoring, and quality control of these assessments, implementing additional quality checks and managing two research assistants in this work.

For the neuroimaging, I designed the thesis protocol and was responsible for developing and managing the 2-3 year old MRI scans alongside Kirsty Donald. This included working with the radiographers to optimise the imaging protocol; working with a sleep management specialist to improve the sleep success rate; training the research nurses; setting up a formal clinical reporting system; co-ordinating the participant recruitment and transport logistics from Paarl to Groote Schuur Hospital; and overseeing the imaging sessions and dedicated team that worked tirelessly to scan the children. I worked with Sivenesi Subramoney to do the MRI processing with support from Jean-Paul Fouche and UCLA advisers, Katherine Narr and Shantanu Joshi. For the neonatal scans, I was not involved in the data collection which occurred prior to the PhD thesis. I carried out imaging data cleaning, processing and quality review supervised by Nynke Groenewold.

For all results chapters in this thesis, I designed and performed the analyses and interpretation with statistical guidance from Andrea Rehman, support from the DCHS data team, my supervisors, and imaging support from UCLA. I led all the manuscript writing for papers included in the chapters of this thesis. Finally, I supervised Freddy Green, LSHTM MSc student, and Cesc Bertran-Cobo, University of Amsterdam MSc student, and contributed intellectual input to their study design, interpretation and the conversions of their MSc theses to manuscripts (Appendix XI and XII respectively).

3.10 Statement of funding

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Part II

Neurodevelopment

4

Chapter 4: Neurodevelopment of HIV-Exposed Uninfected Children in South Africa: Outcomes from an Observational Birth Cohort Study

(Research paper)

Neurodevelopment of HIV-exposed uninfected children in South Africa: Outcomes from an observational birth cohort study

Summary

Chapter 4 presents the first research paper titled 'Neurodevelopment of HIV-exposed uninfected children in South Africa: outcomes from an observational birth cohort study'. This paper provides an analysis of the cognitive, language, and motor outcomes of children who are HEU compared to HU in the Drakenstein Child Health Study (DCHS) during the first two years of life (objective 1). It also examines associations between neurodevelopment and maternal CD4 cell count in pregnancy (secondary objective). A directed acyclic graph (DAG) was developed, guided by the literature on child development, that informed the multivariable analyses in this paper as well as others in the thesis.

While there were no group differences in neurodevelopment at age 6 months, uninfected children exposed to maternal HIV infection had impaired language development compared to unexposed children by two years of age, with increased odds of receptive and expressive language delays. No significant differences were found between HEU and HU children in other developmental domains. Further, maternal immunosuppression (as defined by CD4 cell count in pregnancy ≤500 cells/microlitre) was associated with poorer language outcomes. Overall, the results suggest that HEU children may be at higher risk of language impairment, and the results are identifiable as early as two years of age. The CD4 results suggest a potential immune-related mechanism, and highlight the importance of optimising maternal health for child neurodevelopmental outcomes.

My role in this work involved designing the research question, contributing to the data collection and leading the analysis and write-up. This included monitoring and quality control of the neurodevelopmental assessments following completion of a Bayley Scales of Infant & Toddler Development-III course, with oversight from Prof. Kirsty Donald (supervisor); managing two research assistants; working with the DCHS data team and study doctor at Paarl Hospital to collect, process and quality control baseline demographic and HIV-related data; conceiving and conducting the data analysis under the supervision of A/Prof. Andrea Rehman (statistician) and Raymond Nhapi (data analyst); interpretation of results; and writing the first manuscript draft, incorporating feedback from co-authors and peer review.

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Figure 4.2: Directed acyclic graph

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Supplementary material

The supplementary material as detailed in the published article is available at https://doi.org/10.1016/S2352-4642(19)30250-0 and listed in Appendix V.

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SECTION A – Student Details

Student ID Number	383999	**Title**	Dr
First Name(s)	Catherine		
Surname/Family Name	Scrymgeour-Wedderburn		
Thesis Title	Neurodevelopmental effects of HIV exposure: a prospective neuroimaging study of uninfected children born to mothers living with HIV		
Primary Supervisor	Professor Shunmay Yeung		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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SECTION E

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Neurodevelopment of HIV-exposed uninfected children in South Africa: outcomes from an observational birth cohort study

Catherine J Wedderburn, Shunmay Yeung, Andrea M Rehman, Jacob A M Stadler, Raymond T Nhapi, Whitney Barnett, Landon Myer, Diana M Gibb, Heather J Zar, Dan J Stein, Kirsten A Donald

Summary

Background HIV infection is known to cause developmental delay, but the effects of HIV exposure without infection during pregnancy on child development are unclear. We compared the neurodevelopmental outcomes of HIV-exposed uninfected and HIV-unexposed children during their first 2 years of life.

Methods Pregnant women (>18 years of age) at 20–28 weeks' gestation were enrolled into the Drakenstein Child Health cohort study while attending routine antenatal appointments at one of two peri-urban community-based clinics in Paarl, South Africa. Livebirths born to enrolled women during follow-up were included in the birth cohort. Mothers and infants received antenatal and postnatal HIV testing and antiretroviral therapy per local guidelines. Developmental assessments on the Bayley Scales of Infant and Toddler Development, third edition (BSID-III), were done in a subgroup of infants at 6 months of age, and in the full cohort at 24 months of age, with assessors masked to HIV exposure status. Mean raw scores and the proportions of children categorised as having a delay (scores <–2 SDs from the reference mean) on BSID-III were compared between HIV-exposed uninfected and HIV-unexposed children.

Findings 1225 women were enrolled between March 5, 2012, and March 31, 2015. Of 1143 livebirths, 1065 (93%) children were in follow-up at 6 months and 1000 (87%) at 24 months. Two children were diagnosed with HIV infection between birth and 24-month follow-up and were excluded from the analysis. BSID-III assessments were done in 260 (24%) randomly selected children (61 HIV-exposed uninfected, 199 HIV-unexposed) at 6 months and in 732 (73%) children (168 HIV-exposed uninfected, 564 HIV-unexposed) at 24 months. All HIV-exposed uninfected children were exposed to antiretrovirals (88% to maternal triple antiretroviral therapy). BSID-III outcomes did not significantly differ between HIV-exposed uninfected and HIV-unexposed children at 6 months. At 24 months, HIV-exposed uninfected children scored lower than HIV-unexposed for receptive language (adjusted mean difference –1·03 [95% CI –1·69 to –0·37]) and expressive language (–1·17 [–2·09 to –0·24]), whereas adjusted differences in cognitive (–0·45 [–1·32 to 0·43]), fine motor (0·09 [–0·49 to 0·66]), and gross motor (–0·41 [–1·09 to 0·27]) domain scores between groups were not significant. Correspondingly, the proportions of HIV-exposed uninfected children with developmental delay were higher than those of HIV-unexposed children for receptive language (adjusted odds ratio 1·96 [95% CI 1·09 to 3·52]) and expressive language (2·14 [1·11 to 4·15]).

Interpretation Uninfected children exposed to maternal HIV infection and antiretroviral therapy have increased odds of receptive and expressive language delays at 2 years of age. Further long-term work is needed to understand developmental outcomes of HIV-exposed uninfected children, especially in regions such as sub-Saharan Africa that have a high prevalence of HIV exposure among children.

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Introduction

More than 1·4 million children are born to HIV-infected mothers annually, and 90% live in sub-Saharan Africa.[1] However, following the success of programmes for the prevention of mother-to-child transmission of HIV through maternal antiretroviral therapy (ART), most children born to HIV-infected mothers are not infected with HIV, and there are an estimated 14·8 million HIV-exposed uninfected children worldwide.[1] Whereas paediatric HIV infection is known to delay neurodevelopment,[2] the outcomes of HIV-exposed uninfected children are less clear.

HIV-exposed uninfected children have increased morbidity and mortality,[3] and might also have adverse developmental outcomes compared with HIV-unexposed children. Several studies have described varying degrees of impaired cognitive, language, and motor development in HIV-exposed uninfected children, particularly in low-resource settings,[4–6] including South Africa.[7] However, other studies have found no substantial evidence of developmental delay.[8,9] Few studies have investigated children exposed to current first-line antiretroviral drug therapy in sub-Saharan Africa, and most have not

Research in context

Evidence before this study

We searched six external databases (MEDLINE, PubMed, Embase, PsychINFO, Africa-Wide Information, and Global Health) for articles published from database inception until April 30, 2019, that examined the neurodevelopment of HIV-exposed uninfected children. The search terms used included those related to the concepts of "child", "neurodevelopment", and "HIV / antiretroviral (ARV) drugs", which were adapted for use with the different databases and combined with database-specific filters where available. We excluded studies published before the year 2000. Longitudinal studies from high HIV-burden countries in the current era of antiretroviral therapy (ART) are scarce, as highlighted by reviews. Many previous studies have had small sample sizes and lacked adequate comparison groups or assessment of potential confounders. Overall, there is growing recognition that HIV-exposed uninfected children might have poorer developmental outcomes compared with HIV-unexposed children, particularly in low-income and middle-income countries. Several studies have reported impairments in cognitive, language, or motor function. Other studies, including one from Uganda and Malawi, did not find substantial differences between HIV-exposed uninfected and HIV-unexposed children. Given that most studies have been cross-sectional and that not all data are consistent, the exact nature of developmental delay and the clinical relevance remain unclear.

Added value of this study

This population-based study investigates the effect of HIV and antiretroviral exposure on early child development in a well characterised South African cohort in the current ART era. We found no difference between HIV-exposed uninfected and HIV-unexposed children from the same environment at 6 months in any developmental domain in our cohort. However, by 24 months, HIV-exposed uninfected children had significantly poorer receptive and expressive language outcomes and had increased risks of delay (scores <–2 SD from the reference mean of Bayley Scales of Infant and Toddler Development, third edition) in these domains compared with HIV-unexposed children after controlling for relevant confounders. Additionally, in an exploratory analysis, we found an association between language delay and maternal immunosuppression. Cognitive and motor outcomes at 24 months were not significantly affected by HIV exposure.

Implications of all the available evidence

HIV-exposed uninfected children might be at higher risk of delayed language development (both receptive and expressive) compared with HIV-unexposed children, and these delays are identifiable as early as 24 months of age. Consistently, previous studies from both high-resource and low-resource settings have suggested an association between HIV exposure without infection and adverse language outcomes. The increased severe language delay is concerning, and further follow-up of these children is needed to ascertain whether the effects have a continued impact later in life and to delineate the potential mechanisms. Identifying those children who are most susceptible to poor developmental outcomes is necessary to focus interventions and improve child health outcomes. Given that almost a quarter of South African children are exposed to HIV, and that numbers of HIV-exposed uninfected children are increasing globally, these findings could have important implications for public health policies.

documented infant feeding mode, which has been associated with neurodevelopment.[7] Additionally, many previous studies have had small sample sizes or cross-sectional design, or have lacked adequate HIV-unexposed comparison groups or assessment of potential confounders. Given the heterogeneity of studies to date, uncertainty remains regarding the developmental outcomes of HIV-exposed uninfected children.

Neurodevelopment during fetal and early life, a time of intense brain maturation, forms the basis of academic achievement and economic productivity. Identifying the children most susceptible to delays in neurodevelopment is necessary to focus interventions and improve child health outcomes. According to proxy measures of stunting and poverty, sub-Saharan Africa has the highest proportion of children at risk of not reaching their developmental potential worldwide.[10] Therefore, understanding whether neurodevelopment is impaired in the expanding population of HIV-exposed uninfected children in this region is important, and studies are needed to ascertain the exact nature of any developmental delay with appropriate controls in the current ART era.[3]

We aimed to compare neurodevelopmental outcomes of HIV-exposed uninfected and HIV-unexposed children from the South African Drakenstein Child Health Study (DCHS) during their first 2 years of life.

Methods

Study design and participants

The DCHS is a population-based birth cohort study based in Paarl (a peri-urban area of the Western Cape, South Africa), investigating the early-life determinants of child health and development.[11,12] The antenatal HIV prevalence in this study population is 21%. Pregnant women who were older than 18 years of age, receiving antenatal care at a participating site, and intending to reside in the area for at least a year were enrolled into the study at 20–28 weeks' gestation while attending routine antenatal appointments.[11,12] Participants were recruited from two community-based clinics: T C Newman clinic (serving a mixed-ancestry community who speak

Afrikaans) and Mbekweni clinic (serving a predominantly black African community who speak isiXhosa), both of which provide free maternal and child care. Written informed consent was obtained annually for mother–child pairs. A subgroup of randomly selected children underwent developmental assessment at 6 months, and all available children were assessed at age 24 months.

This study was approved by the human research ethics committee of the Faculty of Health Sciences, University of Cape Town (approval numbers 401/2009 and 044/2017), and by the London School of Hygiene & Tropical Medicine observational and interventions research ethics committee (approval number 11903).

Procedures

Maternal HIV status during pregnancy was confirmed by routine HIV testing at booking, with retesting of HIV-negative mothers every 12 weeks, in accordance with Western Cape prevention of mother-to-child transmission of HIV guidelines. Additionally, maternal interviews and HIV status reviews of mothers and children were done by study staff at the child's birth, age 6 weeks, and every 6 months thereafter. All HIV-infected mothers were initiated on antiretroviral drugs according to prevention of mother-to-child transmission guidelines at the time: three-drug ART (the first-line ART regimen consisted of two nucleoside reverse transcriptase inhibitors plus a non-nucleoside reverse transcriptase inhibitor, commonly tenofovir plus emtricitabine plus efavirenz) or zidovudine from 14 weeks' gestation, depending on maternal clinical and immunological status (before May, 2013); or ART for life for all pregnant women (from May, 2013). All HIV-exposed uninfected children received prophylaxis (nevirapine alone or combined with zidovudine) from birth. HIV and ART data were collected by triangulating clinic and hospital folder information and maternal self-report interviews. Maternal CD4 cell count and viral load data were obtained from the online National Health Laboratory Service system. Where there were multiple results, the highest viral load during pregnancy was taken, and the lowest CD4 cell count within 1 year before childbirth and 3 months post-birth was used to maximise numbers. All HIV-exposed children received HIV testing as per local guidelines. HIV detection was done by PCR at age 6 weeks, and by rapid antibody, PCR, or ELISA at age 9 months and 18 months. HIV-exposed uninfected children were confirmed to have a negative HIV test result at age 18 months or a negative test after cessation of breastfeeding if this occurred at more than 18 months of age. HIV-unexposed children were defined as children born to mothers without HIV infection.

Sociodemographic data were collected between weeks 28 and 32 of gestation by trained study staff using structured interviews and questionnaires adapted from the South African Stress and Health study.[11,12]

Detailed birth data were obtained at delivery. Gestational age was calculated using the best estimated delivery date based on antenatal ultrasound, the last menstrual period, or the symphysis-fundal height. Prematurity was defined as birth at less than 37 weeks' gestation. Infant feeding method and exclusive breastfeeding duration were documented by maternal report at age 6–14 weeks, 6 months, and 9 months.

Maternal psychosocial data were collected antenatally between weeks 28 and 32 of gestation.[12] Maternal alcohol use during pregnancy was assessed using the Alcohol, Smoking, and Substance Involvement Screening Test.[12] Material tobacco exposure during pregnancy was assessed by use of the IMMULITE 1000 nicotine metabolite kit (Siemens Medical Solutions Diagnostics, Glyn Rhonwy, UK) to measure antenatal urine cotinine concentration. Maternal depression was assessed with the Edinburgh Postnatal Depression Scale (with a score of ≥13 considered to indicate depression).[12]

Developmental assessment

Neurodevelopment was measured with the Bayley Scales of Infant and Toddler Development, third edition (BSID-III),[13,14] which is widely used internationally and has been validated in South Africa with reported values similar to those of the BSID-III US-based reference population.[15,16] The objectively measured assessment consists of five subscales: cognition, receptive language, expressive language, fine motor, and gross motor. Trained assessors masked to HIV exposure status alternated testing between clinics, assessing equal numbers of children at each site and offering language prompts in the child's preferred language. Any child with significant developmental delay was referred to the relevant health-care service. Inter-assessor reliability was assured through training and supervision. Assessors were monitored by a paediatric neurodevelopmental specialist throughout, who periodically observed assessments to ensure standardised data collection across sites, accuracy, and continued agreement between assessors. A second level of external quality control was done centrally before data capture. Scores were entered into a specialised BSID-III software programme.

260 children were randomly selected from the original cohort as a sampling frame for developmental assessment at 6 months of age. If a mother–child pair was unavailable, another child aged 6 months was invited to attend from those with study follow-up visits that week, based on child age at the time (a convenience sample). All available children were assessed at age 24 months. Mothers who were unavailable at initial contact were tried again up to three times, and children who were unwell on the day of assessment were rebooked where possible. Raw scores, scaled scores, and developmental delay are reported. Raw scores represent the sum of individual items the child passes on each subscale. Raw scores were converted to age-adjusted scaled scores standardised using normative data derived from a US reference population, with a range of 1–19 and mean of 10 (SD 3), with correction for

prematurity at 6 months.[13] A BSID-III score of less than –2 SDs from the BSID-III reference mean was used to define a clinically significant delay in any domain.[7,13]

Statistical analysis

Maternal and child sociodemographic characteristics were expressed as mean (SD) for continuous data or absolute frequencies (%) for categorical data, and were compared between HIV-exposed uninfected and HIV-unexposed children with BSID-III results at 6 months and 24 months using descriptive statistics (*t* tests or χ^2 tests).

The associations between HIV exposure and each BSID-III subscale (cognitive, receptive language, expressive language, fine motor, and gross motor) at 6 months and 24 months of age were compared with use of linear regression models for raw scores and logistic regression models for developmental delay. Standardised effect sizes were reported as Cohen's d values. A directed acyclic graph (DAG) was constructed using DAGitty, according to previously published literature, to delineate assumptions regarding the causal pathway between HIV exposure and neurodevelopment. Multivariable models were then created using all potential confounders of the exposure–outcome relationship, as determined a priori by the DAG (household income, maternal education, maternal age, and sex and age of child). Residuals were checked for normality through quantile-quantile plots to confirm the linearity assumption for the models. Adjusted mean differences, adjusted odds ratios (ORs), and 95% CIs are presented.

For more on **DAGitty** see http://dagitty.net/

Additional analyses were done by adjusting the models for key potential mediating variables identified from the DAG (prematurity, maternal depression, and breastfeeding [exclusive breastfeeding duration and exclusive breastfeeding to 6 months]) to assess any changes to specific domain associations. Sensitivity analyses were done to examine the effect of site and home language in a restricted subanalysis of Mbekweni clinic, where the majority of HIV-exposed uninfected children reside. A separate analysis limited to HIV-exposed uninfected children whose mothers were on first-line ART was also done. Finally, the association between maternal CD4 cell count (dichotomised into ≤500 and >500 cells per µL, as per previous guidelines,[17] with similar group sizes) and developmental outcomes was explored in BSID-III language subscales to provide direction for future work.

Statistical analyses were done with STATA software (version 14.0). P values of less than 0·05 (two-tailed) were considered to indicate statistical significance.

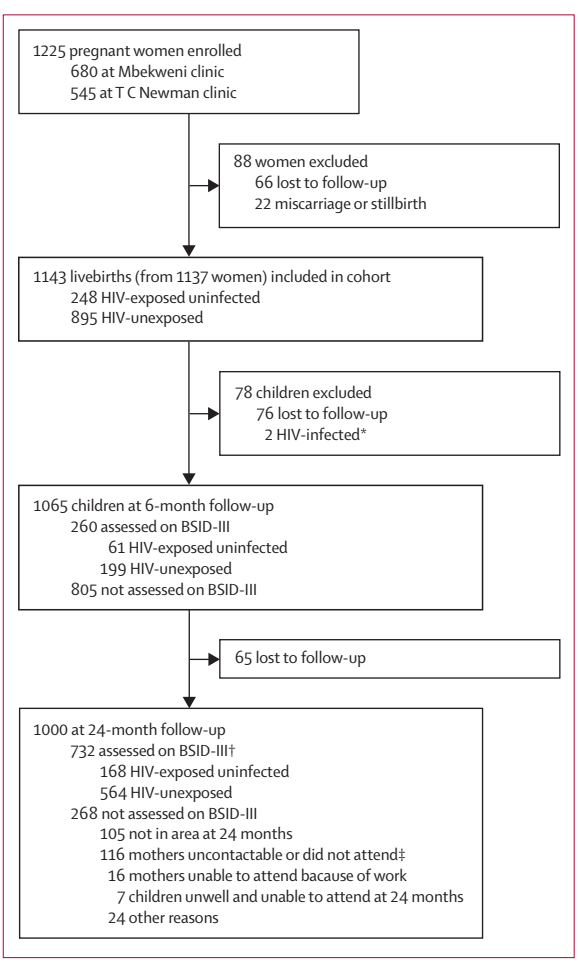

Figure 1: **Drakenstein Child Health Study profile**
BSID-III=Bayley Scales of Infant and Toddler Development, third edition. *Excluded from this analysis, but not from the Drankenstein Child Health Study follow-up. †209 (80%) of the 260 children assessed on BSID-III at 6 months were also assessed at 24 months (46 HIV-exposed uninfected and 163 HIV-unexposed). ‡No show because of violence, poor weather conditions, or seasonal work.

Role of the funding source

The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between March 5, 2012, and March 31, 2015, 1225 pregnant women were enrolled into the study. Of 1143 live infants born to 1137 enrolled women, follow-up was available for 1065 (93%) children at 6 months and 1000 (87%) at 24 months of age, excluding two children with HIV infection identified between birth and 24-month follow-up (figure 1). BSID-III assessments were done for a subgroup of 260 (24%) uninfected children (61 HIV-exposed uninfected and 199 HIV-unexposed) at 6 months and for 732 (73%) children (168 HIV-exposed uninfected and 564 HIV-unexposed) at 24 months. Demographic characteristics of HIV-exposed uninfected and HIV-unexposed children at 6 months and 24 months were similar (table 1). Mothers with and mothers without HIV infection did not differ significantly in terms of household income, employment status, marital status, maternal depression, or alcohol use during pregnancy. Similar proportions of HIV-exposed uninfected and

	6 months			24 months		
	HIV-exposed (N=61)	HIV-unexposed (N=199)	p value	HIV-exposed (N=168)	HIV-unexposed (N=564)	p value
Child age at BSID-III assessment, days	184 (11)	184 (12)	0·84	732 (16)	733 (16)	0·53
Sex	..	..	0·70	..	..	0·20
Male	33/61 (54%)	102/199 (51%)	..	94/168 (56%)	284/564 (50%)	..
Female	28/61 (46%)	97/199 (49%)	..	74/168 (44%)	280/564 (50%)	..
Site (proxy for primary language)	..	..	<0·0001	..	..	<0·0001
T C Newman clinic (Afrikaans)	3/61 (5%)	130/199 (65%)		13/168 (8%)	331/564 (59%)	..
Mbekweni clinic (isiXhosa)	58/61 (95%)	69/199 (35%)		155/168 (92%)	233/564 (41%)	..
Monthly household income, South African rand*	..	..	0·74	..	..	0·93
<1000	26/61 (43%)	88/199 (44%)	..	66/168 (39%)	221/564 (39%)	..
1000–5000	30/61 (49%)	89/199 (45%)	..	83/168 (49%)	273/564 (48%)	..
>5000	5/61 (8%)	22/199 (11%)	..	19/168 (11%)	70/564 (12%)	..
Maternal education	..	..	0·99	..	..	0·0010
Any primary	4/61 (7%)	11/199 (6%)	..	19/168 (11%)	40/564 (7%)	..
Any secondary	34/61 (56%)	115/199 (58%)	..	108/168 (64%)	290/564 (51%)	..
Completed secondary	20/61 (33%)	64/199 (32%)	..	37/168 (22%)	196/564 (35%)	..
Any tertiary	3/61 (5%)	9/199 (5%)	..	4/168 (2%)	38/564 (7%)	..
Maternal death	0	0	..	2/168 (1%)	1/564 (0·2%)	0·071
Mother attended BSID-III assessment	54/60 (90%)	179/198 (90%)	0·93	143/168 (85%)	470/549 (86%)	0·87
Mother in employment	15/61 (25%)	43/199 (22%)	0·63	41/168 (24%)	142/564 (25%)	0·84
Mother married or cohabitating	24/61 (39%)	71/198 (36%)	0·62	75/168 (45%)	217/563 (39%)	0·16
Maternal age at birth, years	29·8 (5·4)	25·5 (5·3)	<0·0001	30·4 (5·3)	26·3 (5·6)	<0·0001
Gestational age at delivery, weeks	38·8 (2·3)	38·7 (2·2)	0·80	38·5 (2·6)	38·6 (2·5), N=562	0·64
Premature birth (<37 weeks' gestation)	9/61 (15%)	24//199 (12%)	0·58	23/168 (14%)	81/562 (14%)	0·81
Birthweight						
Mean (SD), g	3102 (501)	3043 (552)	0·46	3022 (583)	3039 (579)	0·74
Low birthweight (<2·5 kg)	7/61 (11%)	25/199 (13%)	0·82	23/168 (14%)	84/564 (15%)	0·70
Birth length, cm	49·5 (4·3)	49·8 (3·7), N=198	0·60	49·9 (4·1), N=165	49·9 (3·6), N=555	0·94
Birth head circumference, cm	33·5 (1·8)	33·5 (1·9), N=198	0·88	33·6 (2·1), N=167	33·5 (2·1), N=558	0·72
WHO length/height-for-age Z-score	−0·44 (1·5), N=59	−0·44 (1·7), N=189	0·98	−1·17 (1·2), N=130	−1·09 (1·2), N=449	0·49
Maternal smoking during pregnancy (urine cotinine concentration)†	..	..	0·010	..	..	0·10
Active (≥500 ng/mL)	14/61 (23%)	87/195 (45%)	..	46/164 (28%)	204/550 (37%)	..
Passive (10–500 ng/mL)	31/61 (51%)	71/195 (36%)	..	79/164 (48%)	229/550 (42%)	..
Non-smoker (<10 ng/mL)	16/61 (26%)	37/195 (19%)	..	39/164 (24%)	117/550 (21%)	..
Moderate-to-severe maternal alcohol use during pregnancy	8/55 (15%)	44/192 (23%)	0·18	16/148 (11%)	79/507 (16%)	0·15
Maternal depression during pregnancy	9/55 (16%)	57/191 (30%)	0·047	33/149 (22%)	123/508 (24%)	0·60
Exclusive breastfeeding duration, months						
Mean (SD)	1·1 (2·0)	2·4 (2·0)	<0·0001	1·5 (2·1)	2·3 (1·9), N=563	<0·0001
6 months‡	7/61 (11%)	37/199 (19%)	0·20	24/168 (14%)	100/563 (18%)	0·29
Maternal HIV diagnosis timepoint						
Before pregnancy	44/59 (75%)	..	..	122/163 (75%)	..	..
During pregnancy	15/59 (25%)	..	..	41/163 (25%)	..	..
Maternal CD4 cell count in pregnancy, cells/μL						
Median (range)	522 (298–691), N=56	..	..	441 (294–618), N=151	..	..
<200	6/56 (11%)	..	..	17/151 (11%)	..	..
200–350	12/56 (21%)	..	..	37/151 (25%)	..	..
350–500	9/56 (16%)	..	..	33/151 (22%)	..	..
>500	29/56 (52%)	..	..	64/151 (42%)	..	..

(Table 1 continues on next page)

	6 months			24 months		
	HIV-exposed (N=61)	HIV-unexposed (N=199)	p value	HIV-exposed (N=168)	HIV-unexposed (N=564)	p value
(Continued from previous page)						
Highest maternal viral load during pregnancy						
Below detectable limit (<40 copies/mL)	25/36 (69%)	..	..	69/108 (64%)	..	..
Detectable (≥40–1000 copies/mL)	6/36 (17%)	..	..	25/108 (23%)	..	..
Unsuppressed (>1000 copies/mL)	5/36 (14%)	..	..	14/108 (13%)	..	..
Antiretroviral drug initiation						
Before pregnancy	22/59 (37%)	..	..	71/165 (43%)	..	..
During pregnancy	37/59 (63%)	..	..	94/165 (57%)	..	..
Antiretroviral regimen during pregnancy						
Prevention of mother-to-child transmission prophylaxis (zidovudine)	9/58 (16%)	..	..	20/163 (12%)	..	..
First-line triple therapy§	48/58 (83%)	..	..	132/163 (81%)	..	..
Second-line or third-line therapy	1/58 (2%)	..	..	11/163 (7%)	..	..
Infant prophylaxis						
Nevirapine alone	55/60 (92%)	..	..	145/167 (87%)	..	..
Nevirapine and zidovudine	5/60 (8%)	..	..	22/167 (13%)	..	..

Data are n/N (%), mean (SD), or median (IQR). Continuous variables were compared with unpaired t tests; categorical variables were compared with χ^2 tests. All percentages calculated on non-missing values. N values are indicated where the number of participants with available data differs from the total group size. Missing data: birth head circumference (n=1 at 6 months, n=7 at 24 months); birth length (n=1 at 6 months, n=12 at 24 months); birthweight (n=1 at 24 months); WHO length/height-for-age Z score (n=12 at 6 months, n=153 at 24 months); gestation delivery (n=2 at 24 months); breastfeeding duration (n=1 at 24 months). Maternal CD4 taken as the lowest CD4 from 1 year before to 3 months after delivery to reflect maternal immunosuppression during pregnancy with the highest sample size. BSID-III=Bayley Scales of Infant and Toddler Development, third edition. *1000 South African rand is approximately equal to US$75. †p=0·22 (6 months) and p=0·50 (24 months) for non-smoking versus active and passive smoking together. ‡Exclusive breastfeeding to 6 months was defined as exclusive breastfeeding for >5 months of age. §A non-nucleoside reverse-transcriptase inhibitor backbone and two nucleoside reverse transcriptase inhibitors, most commonly efavirenz with tenofovir and emtricitabine as a fixed-dose combination, although some mothers received nevirapine-based treatment; of those mothers on first-line triple antiretroviral therapy, 42 (88%) at 6 months and 116 (88%) at 24 months received efavirenz-based therapy. No mothers in the HIV-unexposed group were taking any antiretrovirals during pregnancy.

Table 1: **Demographic characteristics of children assessed on BSID-III at 6 months and 24 months according to HIV exposure**

	HIV-exposed		HIV-unexposed		Unadjusted			Adjusted*		
	N	Mean raw score (SD)	N	Mean raw score (SD)	Mean difference (95% CI)	p value	Effect size, Cohen's d (95% CI)	Mean difference (95% CI)	p value	Effect size, Cohen's d (95% CI)
6 months										
Cognitive	60	27·55 (2·82)	196	27·08 (3·69)	0·47 (–0·55 to 1·49)	0·37	0·13 (–0·16 to 0·42)	0·69 (–0·33 to 1·72)	0·19	0·20 (–0·09 to 0·49)
Receptive language	60	9·78 (1·55)	194	9·57 (1·65)	0·22 (–0·26 to 0·69)	0·37	0·13 (–0·16 to 0·42)	0·23 (–0·28 to 0·73)	0·38	0·14 (–0·15 to 0·43)
Expressive language	61	8·62 (2·72)	194	8·89 (2·61)	–0·26 (–1·03 to 0·50)	0·50	–0·10 (–0·39 to 0·19)	–0·42 (–1·22 to 0·39)	0·31	–0·16 (–0·45 to 0·13)
Fine motor	61	21·79 (2·85)	196	21·60 (3·03)	0·19 (–0·67 to 1·05)	0·67	0·06 (–0·22 to 0·35)	0·55 (–0·32 to 1·42)	0·22	0·19 (–0·10 to 0·47)
Gross motor	61	24·75 (3·73)	195	24·54 (3·66)	0·21 (–0·85 to 1·27)	0·70	0·06 (–0·23 to 0·34)	0·57 (–0·48 to 1·62)	0·29	0·16 (–0·13 to 0·44)
24 months										
Cognitive	167	54·84 (5·06)	562	55·69 (4·73)	–0·85 (–1·68 to –0·02)	0·045	–0·18 (–0·35 to –0·003)	–0·45 (–1·32 to 0·43)	0·32	–0·09 (–0·27 to 0·08)
Receptive language	165	19·83 (3·54)	556	21·10 (3·72)	–1·27 (–1·91 to –0·63)	0·0001	–0·34 (–0·51 to –0·16)	–1·03 (–1·69 to –0·37)	0·0024	–0·28 (–0·45 to –0·10)
Expressive language	158	22·91 (5·37)	542	24·45 (4·94)	–1·54 (–2·43 to –0·64)	0·0008	–0·30 (–0·48 to –0·12)	–1·17 (–2·09 to –0·24)	0·013	–0·23 (–0·41 to –0·05)
Fine motor	166	37·40 (3·34)	562	37·51 (3·10)	–0·13 (–0·68 to 0·42)	0·64	–0·04 (–0·21 to 0·13)	0·09 (–0·49 to 0·66)	0·77	0·03 (–0·15 to 0·20)
Gross motor	159	53·07 (3·37)	535	53·31 (3·66)	–0·24 (–0·88 to 0·39)	0·46	–0·07 (–0·24 to 0·11)	–0·41 (–1·09 to 0·27)	0·24	–0·11 (–0·29 to 0·06)

Residuals were assessed for each model using quantile–quantile plots and were normally distributed. Negative mean difference estimates indicate that HIV exposure was associated with lower total raw scores in that BSID-III domain (ie, poorer outcomes). BSID-III=Bayley Scales of Infant and Toddler Development, third edition. *Adjusted for child age, child sex, maternal education, household income, and maternal age.

Table 2: **Unadjusted and adjusted mean differences in BSID-III domain raw scores at 6 months and 24 months according to HIV exposure**

HIV-unexposed children were born premature or with low birthweight. Most children attended the BSID-III assessment with their mother. Exclusive breastfeeding was uncommon, with less than 20% of mothers exclusively breastfeeding for 6 months. Among children who exclusively breastfed, breastfeeding duration was shorter for HIV-exposed uninfected than for HIV-unexposed children. Overall, a higher proportion of children seen at the Mbekweni clinic were HIV-exposed uninfected than were children seen at the T C Newman clinic. HIV-infected mothers were on average older at the time of childbirth than were uninfected mothers.

Among children assessed at 24 months, HIV-infected mothers had lower educational attainment than did uninfected mothers. Median maternal CD4 was 441 cells per µL, and 69 (64%) of those with available results had an undetectable viral load. Maternal antiretroviral drug regimens included zidovudine prophylaxis for prevention of mother-to-child transmission (12%), first-line three-drug ART (81%), and second-line or third-line protease inhibitor-containing therapies (7%; appendix p 1). 43% of women initiated antiretrovirals before pregnancy, and 88% of mothers on first-line ART had efavirenz-based therapy. Children received post-exposure prophylaxis with nevirapine alone (87%), or nevirapine and zidovudine (13%).

The children with BSID-III outcomes were largely representative of the full cohort at each timepoint (appendix p 2), although at 24 months they had a higher maternal age, a higher proportion of maternal alcohol use, and a lower proportion of premature birth. The highest percentage of missing data in any single BSID-III domain was 5%.

At 6-month BSID-III assessments, HIV-exposed uninfected and HIV-unexposed children showed no significant differences in mean raw scores on any of the subscales (table 2). Mean scaled scores for all subscales were within 1 SD of the BSID-III reference mean (mean score 10 [SD 3]; appendix p 4). Numbers of children with developmental delay were low across the cohort (two to seven children in any subscale).

At 24 months, univariate analysis of BSID-III cognition outcomes showed that HIV-exposed uninfected children had lower mean raw scores than did HIV-unexposed children (mean difference –0·85 [95% CI –1·68 to –0·02]). However, this difference was attenuated after adjustment for confounding variables (–0·45 [–1·32 to 0·43]; table 2). The proportions of children with developmental delay (defined as a scaled score less than –2 SDs from the BSID-III reference mean) on the cognition subscale were similar between HIV-exposed uninfected and HIV-unexposed children (11% *vs* 9%, adjusted OR 1·01 [95% CI 0·55 to 1·85]; table 3).

In both unadjusted and adjusted models, HIV exposure was associated with lower 24-month scores for receptive language (adjusted mean difference –1·03 [–1·69 to –0·37]) and expressive language (adjusted mean difference –1·17 [–2·09 to –0·24]; table 2). Although the effect sizes were small (adjusted Cohen's d –0·28 [95% CI –0·45 to –0·10] for receptive and –0·23 [–0·41 to –0·05] for expressive language), these differences represent reduced ability to understand the meaning of words and commands

	Infants with delay, n/N (%)		Unadjusted		Adjusted*	
	HIV-exposed	HIV-unexposed	OR (95% CI)	p value	OR (95% CI)	p value
Cognitive	18/167 (11%)	52/562 (9%)	1·18 (0·67 to 2·09)	0·56	1·01 (0·55 to 1·85)	0·97
Receptive language	23/165 (14%)	40/556 (7%)	2·09 (1·21 to 3·61)	0·0081	1·96 (1·09 to 3·52)	0·025
Expressive language	18/158 (11%)	31/542 (6%)	2·12 (1·15 to 3·90)	0·016	2·14 (1·11 to 4·15)	0·024
Fine motor	6/166 (4%)	12/562 (2%)	1·72 (0·64 to 4·65)	0·29	1·53 (0·53 to 4·42)	0·44
Gross motor	6/159 (4%)	19/535 (4%)	1·07 (0·42 to 2·71)	0·90	1·23 (0·44 to 3·43)	0·69

We did a complete case analysis by outcome. ORs greater than 1 indicate that HIV exposure was associated with higher risk of delay in that BSID-III domain (ie, poorer outcomes). Total number of participants assessed (N) for each domain was the same for both unadjusted and adjusted models; no covariates had missing data. BSID-III=Bayley Scales of Infant and Toddler Development, third edition. OR=odds ratio. *Adjusted for child age, child sex, maternal education, household income, and maternal age.

Table 3: **Odds of developmental delay by BSID-III domain at 24 months according to HIV exposure**

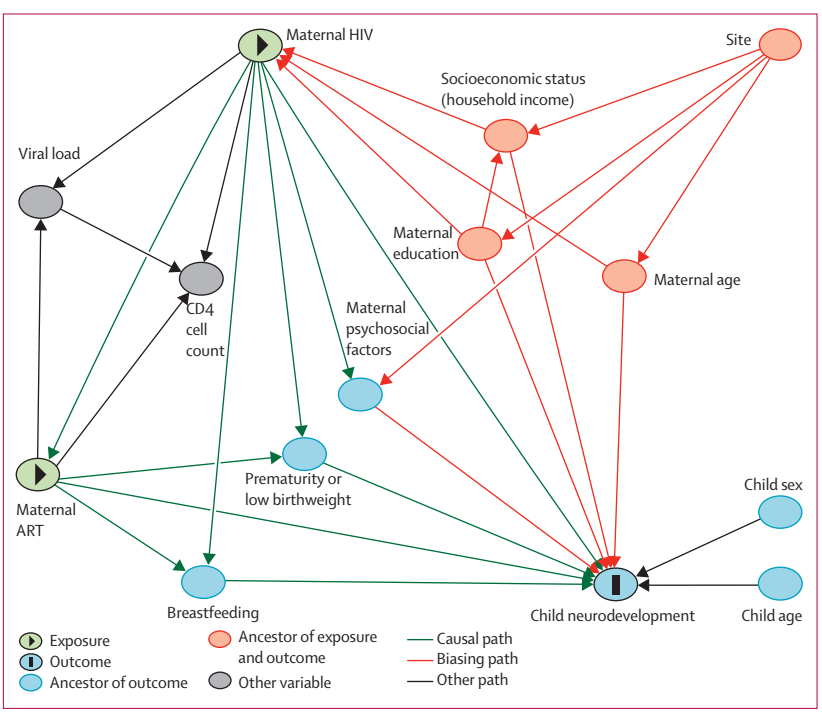

Figure 2: **Directed acyclic graph**

We constructed a directed acyclic graph using DAGitty to examine for possible confounding in the relationship between HIV and ART exposure and child developmental performance on the Bayley Scales of Infant and Toddler Development, third edition, at 6 months and 24 months in the Drakenstein Child Health Study, using multiple sources.[3,10–12] In this model, site acts as a proxy for home language and ethnicity. Maternal psychosocial factors include maternal depression, alcohol use, and smoking. Minimal sufficient adjustment sets for estimating the total effect of maternal HIV and ART exposure on child neurodevelopment include socioeconomic status (household income), maternal education, and maternal age. ART=antiretroviral therapy.

See **Online** for appendix

	N	Raw scores					Delayed development				
		Mean (SD)	Unadjusted		Adjusted*		Delayed, N (%)	Unadjusted		Adjusted*	
			Mean difference (95% CI)	p value	Mean difference (95% CI)	p value		OR (95% CI)	p value	OR (95% CI)	p value
Receptive language											
HIV-unexposed	556	21·10 (3·72)	0·00 (ref)	..	0·00 (ref)	..	40 (7%)	1·00 (ref)	..	1·00 (ref)	..
Maternal CD4 count >500 cells per µL	62	20·35 (3·24)	−0·74 (−1·71 to 0·22)	0·13	−0·48 (−1·45 to 0·49)	0·33	7 (11%)	1·64 (0·70 to 3·84)	0·25	1·56 (0·65 to 3·73)	0·32
Maternal CD4 count ≤500 cells per µL	86	19·27 (3·70)	−1·83 (−2·67 to −0·99)	<0·0001	−1·54 (−2·40 to −0·68)	0·0005	14 (16%)	2·51 (1·30 to 4·84)	0·0061	2·40 (1·19 to 4·83)	0·014
Expressive language											
HIV-unexposed	542	24·45 (4·94)	0·00 (ref)	..	0·00 (ref)	..	31 (6%)	1·00 (ref)	..	1·00 (ref)	..
Maternal CD4 count >500 cells per µL	60	23·87 (5·04)	−0·58 (−1·92 to 0·76)	0·40	−0·16 (−1·50 to 1·18)	0·82	5 (8%)	1·50 (0·56 to 4·01)	0·42	1·42 (0·52 to 3·89)	0·50
Maternal CD4 count ≤500 cells per µL	81	22·09 (5·47)	−2·36 (−3·53 to −1·19)	<0·0001	−1·92 (−3·12 to −0·72)	0·0018	11 (14%)	2·59 (1·25 to 5·38)	0·011	2·73 (1·24 to 6·03)	0·013

ORs greater than 1 indicate higher risk of delay in that BSID-III domain (ie, poorer outcomes) versus the reference group; negative mean difference estimates indicate lower total raw scores in that domain (ie, poorer outcomes) versus the reference group. Maternal CD4 taken as the lowest CD4 from 1 year before to 3 months after delivery to reflect maternal immunosuppression during pregnancy with the highest sample size. OR=odds ratio. BSID-III=Bayley Scales of Infant and Toddler Development, third edition. *Adjusted for child age, child sex, maternal education, household income, and maternal age.

Table 4: **Receptive and expressive language outcomes on BSID-III at 24 months according to maternal CD4 cell counts**

in receptive language, and reduced ability to use sounds and words to communicate in expressive language. Correspondingly, greater proportions of HIV-exposed uninfected than HIV-unexposed children had delayed development in receptive language (14% *vs* 7%, adjusted OR 1·96 [1·09 to 3·52]) and expressive language (11% *vs* 6%, 2·14 [1·11 to 4·15]; table 3).

There were no differences in mean raw scores or the proportions of children with developmental delays between HIV-exposed uninfected and HIV-unexposed children with regard to fine motor or gross motor domains on 24-month BSID-III assessment (tables 2, 3).

In sensitivity analyses adjusting separately for exclusive breastfeeding, premature birth, and maternal depression (factors identified to potentially be on the causal pathway between HIV exposure and neurodevelopment; figure 2, appendix pp 5–7), HIV exposure remained associated with lower receptive and expressive language raw scores. Two exclusive breastfeeding classifications were examined (exclusive breastfeeding duration and exclusive breastfeeding to 6 months [defined as exclusive breastfeeding for >5 months of age]) and, in both cases, the exposure–outcome relationship remained the same. To address between-group differences in site and home language, we did a restricted analysis of children from Mbekweni clinic (appendix p 8). The overall trends in associations were similar to those of the full sample, the point estimates held, and the proportion of children with delayed development in receptive language and expressive language remained the same, although precision was reduced (which was expected with the smaller sample size). Alcohol exposure (appendix p 9) showed no effect on the associations between receptive or expressive language development and HIV exposure.

In a subgroup analysis comparing only HIV-exposed uninfected children whose mothers were initiated on first-line triple ART (n=132) with HIV-unexposed children, language impairments remained associated with HIV exposure (appendix p 10). In an exploratory analysis comparing outcomes among children born to mothers with and without immunosuppression, a maternal CD4 cell count of 500 cells per µL or less was associated with lower receptive and expressive language scores and increased prevalence of developmental delays in these domains versus children born to mothers without HIV infection (table 4).

Discussion

The results from this South African birth cohort show that HIV-exposed uninfected children had poorer language outcomes at 24 months, but not at 6 months, when compared with HIV-unexposed children. To our knowledge, this is the largest longitudinal study to report delayed language development in both receptive and expressive domains in HIV-exposed uninfected children in South Africa, building on previous literature[18–21] suggesting that language might be impaired in this population.

Overall, children in this cohort showed increased developmental impairment over time. At 6 months, developmental performance was no different between HIV-exposed uninfected and HIV-unexposed children.

More subtle language impairments might not easily be identified at such a young age before explicit verbal communication has developed. Alcock and colleagues also found worsening language outcomes in older (aged 16–30 months *vs* 8–15 months) HIV-exposed uninfected children in Kenya.[18] In our study, by 24 months of age, HIV-exposed uninfected children had language impairment in terms of both raw scores and formal delay categorisation, suggesting clinically significant impairment. Although the effect sizes were small, these findings are concerning in the context of a growing HIV-exposed uninfected population in sub-Saharan Africa. The associations between exposure to HIV and antiretroviral therapy and poorer language outcomes remained after examining infant feeding method, maternal depression, and prematurity as potential mediators. We did not find any associations between exposure to HIV and antiretroviral therapy and cognitive or motor development, similar to the results of studies from Botswana, Uganda, and Malawi.[8,9] However, the BSID-III might underestimate delay, which could also contribute to the lack of differences.[16]

Previous research has found language to be particularly affected in HIV-infected children, and has suggested that language development is also possibly affected in HIV-exposed uninfected children in both high-resource and low-resource settings, supporting our results.[2,18] A study of HIV-exposed uninfected children in the USA found increased risk of language impairments compared with population norms.[19] In the Democratic Republic of the Congo, expressive language (and motor) delays were found in HIV-uninfected preschool children born to mothers with HIV/AIDS.[20] A study in Botswana reported that HIV-exposed uninfected 2-year olds had increased adverse expressive language outcomes,[9] and receptive language was impaired in HIV-exposed uninfected children aged 3 years in Uganda.[21] A recent South African study of 1-year-old children did not find any language delay; however, only expressive language was assessed.[7] It is possible that early exposures only manifest later and that language delay might not be evident as early as 12 months of age, as observed in the current study.

We assessed language using the BSID-III, which measures preverbal communication, vocabulary development, and recognition of common objects and animals. The tasks assessed in children younger than 24 months are more universally applicable than those for older children. The BSID-III has been validated in South Africa in infancy and found to be a culturally appropriate tool.[15,16] In young children (up to 2 years of age), research has shown many similarities in the sequence of language acquisition and vocabulary growth across languages and communities.[22] Despite site differences, the HIV-exposed uninfected and HIV-unexposed groups in the current study had similar socioeconomic backgrounds, and the results of the analysis restricted to children seen at the Mbekweni clinic (isiXhosa-speaking) with nearly 400 children support the main findings (appendix p 8).

The mechanisms for the observed developmental delays require further investigation to understand whether a particular subgroup of HIV-exposed uninfected children might be most susceptible. Our exploratory analyses suggest an effect of maternal immunosuppression on neurodevelopment, with maternal CD4 cell counts of 500 cells per μL or fewer associated with poorer language outcomes than those of unexposed children. Growing evidence indicates that maternal immune activation affects neurodevelopment in utero[23] and neuroinflammation might have an important role in cognitive dysfunction.[24] Previously reported neuroimaging findings from a neonatal subgroup of the DCHS found white matter differences between HIV-exposed uninfected and HIV-unexposed neonates.[25] These CD4 results from our study also support findings from a previous study that showed that maternal viral load might predict poorer developmental outcomes,[8] highlighting the importance of optimising maternal health for child neurodevelopment.

Multiple socioeconomic, environmental, and biological factors influence child neurodevelopment.[10] We adjusted for potential confounders in our analyses; however, the effects of HIV exposure on language development might be mediated through other pathways, including parent–child interaction, that are affected by caregiver physical or psychological health, for which further investigation is needed to inform interventions. Evidence for other possible mechanisms, including antiretroviral neurotoxicity, is lacking, and deciphering the role (if any) of antiretroviral neurotoxicity from HIV exposure is challenging. Antiretrovirals are known to cross the placental barrier and have been linked to mitochondrial toxicity, preterm birth, and biological deficits.[26] In a US cohort study, language outcomes were affected by specific antiretrovirals (including atazanavir associated with late language emergence at 1 year of age and tenofovir with speech impairment at 3 years but not 5 years of age).[19] In Botswana, no effect of monotherapy versus triple ART therapy on developmental outcomes was observed,[27] although efavirenz exposure was associated with lower receptive language scores at 2 years of age compared with non-efavirenz-containing therapies.[28] A study from Uganda and Malawi found no increased developmental risk associated with maternal ART.[8] Our findings were confirmed when analyses were restricted to those infants whose mothers received first-line ART (the majority of whom were receiving efavirenz-based therapy). Careful pharmacovigilance is needed going forwards to monitor potential antiretroviral neurotoxicity.

The strengths of this prospective study include the use of a validated measure of neurodevelopment, as well as the large sample size and inclusion of an appropriate control group from a similar socioeconomic environment. Additionally, the cohort recruitment spanned from 2012 to 2015, with heterogeneity in ART

regimens and maternal immune status, although most mothers were on first-line ART, both of which are representative of populations across sub-Saharan Africa. The cohort had a high prevalence of sociodemographic risk factors, similar to other low-income and middle-income settings, giving this study good generalisability.[12,14] Finally, the study measured neurodevelopment at two timepoints, and follow-up continues for these children. Research in high HIV prevalence settings using longitudinal data is essential to inform global child development research priorities.[29]

This study has some important limitations. First, the sample size at 6 months was small and might have been underpowered to detect a difference at this timepoint. Second, at 24 months, not all children in the cohort received a BSID-III assessment and, although we did sensitivity analyses to explore the effect of potential bias, the possibility of selection bias remains. Third, although the BSID-III is a well recognised tool, there are reliability concerns and further standardisation in sub-Saharan African settings is required; for example, the categorisation of delay is based on scaled scores using normative US data that might not be generalisable to a South African population. However, we reported raw scores with similar patterns to the results based on delay categorisation and compared against a control group, adding validity to our outcomes. Fourth, participants might have had hearing loss that is known to affect language development, and hearing assessments are ongoing. Finally, despite similar backgrounds, there were some differences between the HIV-exposed uninfected and HIV-unexposed groups, including lower proportions of breastfed infants among HIV-exposed uninfected children. However, the prevalence of exclusive breastfeeding was low across the whole population. The multivariable models adjusted for confounders and accounted for potential mediating variables, and, by restricting analyses to the Mbekweni clinic, we attempted to minimise residual confounding.

Elucidating risk factors associated with delayed development could inform effective preventive and intervention strategies. HIV exposure appears to affect emerging language development, which is foundational to school readiness and academic outcomes.[30] As there are an estimated 14·8 million HIV-exposed uninfected children worldwide,[1] this research could have significant public health implications. Furthermore, HIV-exposed uninfected children identified with early developmental delay might benefit from interventions to improve outcomes. Future work is needed to assess if these findings are replicated in cohorts with higher breastfeeding prevalence, and to ascertain their long-term significance. Research is also needed to define the causal pathways behind adverse outcomes in HIV-exposed uninfected children, including the role of maternal psychological and family socioeconomic factors, exposure to HIV, and the effect of ART regimens and initiation timing, to provide future interventions. Neurocognitive follow-up and neuroimaging of these children is ongoing and could add to our understanding of the mechanisms behind the observed developmental delays.[14]

In conclusion, we found uninfected children exposed to maternal HIV and antiretroviral drugs had delayed receptive and expressive language development identifiable as early as 24 months of age, compared with HIV-unexposed children from similar environments. Given that almost a quarter of children in South Africa are HIV-exposed and numbers are expanding globally, further work is needed to understand the long-term developmental outcomes of HIV-exposed uninfected children.

Contributors

CJW assisted with data collection, analysed the study data (with supervision from AMR and RTN), and was responsible for interpretation of results and drafting of the manuscript. KAD was responsible for the developmental assessments and, with SY and DMG, assisted with conception and manuscript revisions. HJZ is the principal investigator of the parent study, and contributed to the current study conceptualisation and manuscript review and edits. DJS and LM contributed to the parent study design and revising the manuscript critically for intellectual content. WB and JAMS managed study operations and data collection. All authors reviewed and approved the final manuscript.

Declaration of interests

We declare no competing interests.

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5

Chapter 5: Growth and Neurodevelopment of HIV-Exposed Uninfected Children: A Conceptual Framework

(Review paper)

Growth and neurodevelopment of HIV-exposed uninfected children: A conceptual framework

Summary

Chapter 5 presents the second research paper titled 'Growth and neurodevelopment of HIV-exposed uninfected children: A conceptual framework'. Following a brief summary of the literature on development outcomes of HEU children, this paper describes a conceptual framework for how exposure to HIV and ART may lead to adverse growth and neurodevelopment. The paper proposes that HEU children may be affected indirectly, through the augmentation of universal risk factors underlying poor development, and directly through HIV/ART-specific pathways, which ultimately converge through common pathogenic mechanisms. Atypical brain development is one hypothesised mechanism leading to poor early child development.

My role included designing and developing the conceptual framework in collaboration with Dr Ceri Evans with input from the other co-authors; researching and leading the section on neurodevelopment; reviewing the section on growth; and co-writing the sections on pathogenesis and improving outcomes, incorporating feedback from co-authors for the manuscript in its entirety.

Note: This review paper briefly cites study results from Chapter 4.

List of Figures

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Primary Supervisor	Professor Shunmay Yeung		

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HIV PATHOGENESIS AND TREATMENT (AL LANDAY AND NS UTAY, SECTION EDITORS)

Growth and Neurodevelopment of HIV-Exposed Uninfected Children: a Conceptual Framework

Catherine J. Wedderburn[1,2] · Ceri Evans[3,4] · Shunmay Yeung[1] · Diana M. Gibb[5] · Kirsten A. Donald[2] · Andrew J. Prendergast[3,4]

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Abstract

Purpose of Review The population of HIV-exposed uninfected (HEU) children is expanding rapidly, and over one million HEU infants are born each year globally. Several recent studies have reported that HEU children, particularly in low- and middle-income countries, are at risk of poor outcomes, including impaired growth and neurodevelopment. However, the reasons for poor clinical outcomes amongst HEU children remain unclear.

Recent Findings We summarise the findings from recent large studies that have characterised growth and neurodevelopment in HEU children, identified risk factors and explored underlying mechanistic pathways. We propose a conceptual framework to explain how exposure to HIV and antiretroviral therapy (ART) may lead to adverse growth and neurodevelopment in uninfected children, and review the available evidence and research gaps.

Summary We propose that HEU children are affected both indirectly, through the augmentation of universal risk factors underlying poor growth and neurodevelopment, and directly through HIV/ART-specific pathways, which ultimately may converge through a series of common pathogenic mechanisms. In the era of universal ART, a better understanding of these pathways is crucial to inform future prevention and intervention strategies.

Keywords HIV-exposed uninfected · Child · Growth · Stunting · Early child development

Introduction

Globally, approximately 1.4 million HIV-infected pregnant women give birth each year [1, 2]. The increased coverage of antiretroviral therapy (ART) for pregnant and breastfeeding women through prevention of mother-to-child transmission (PMTCT) programs has dramatically reduced perinatal and postnatal HIV transmission. Correspondingly, the global population of HIV-exposed uninfected (HEU) children is increasing and, in 2017, was estimated to have reached 14.8 million [3]. This number will continue to increase with improved PMTCT coverage as over one million HEU children are born every year, the majority of whom are now also exposed to ART [3–5]. Emerging data showing poorer health outcomes of HEU compared to HIV-unexposed children [6] means there is a pressing need to understand and address the mechanisms underlying compromised outcomes.

The Sustainable Development Goals have focused attention on the importance of healthy growth and development, so that children can thrive as well as survive. The in utero period and first 2 years after birth (the first 1000 days) represent a highly sensitive period of development, during which substantial physical growth, including brain maturation, occurs [7]. Early fetal exposures are recognised to have long-term consequences for the child

Catherine J. Wedderburn and Ceri Evans contributed equally to this work.

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and future adult. Given the number of children exposed to HIV and ART in utero, it is important to understand the effects of these exposures. This review will first explore growth and development in HEU children; second, outline a conceptual framework to highlight the pathways through which HIV exposure may impact growth and development; and third, examine how this may help to inform prevention and intervention strategies.

Studies of HIV-Exposed Uninfected Children

Over recent decades, there has been a growing number of studies evaluating the outcomes of HEU children. However, interpretation of studies can be challenging for several reasons. First, the effects of HIV exposure may differ between high-income and low-income settings. The epidemiology of HIV infection in high-income settings, where adult infections are often found in populations with more substance use and mental health disorders, differs from the more generalised epidemic in sub-Saharan Africa. In addition, HEU children vary in terms of duration of exposure to both HIV (prenatal/postnatal) and ART and are exposed to different antiretroviral drug classes and combinations. This may mean mechanisms differ across countries. Second, studies are often limited by a lack of comparable HIV-unexposed control groups, particularly due to differences in breastfeeding and socioeconomic status. Third, studies have often been small in size without adequate HIV testing of included children to rule out HIV infection. Fourth, there has been a lack of standardised definitions of outcomes. Despite these limitations, it is possible to draw conclusions on health outcomes from large, well-conducted studies, many of which have clearly demonstrated that HEU children have higher mortality than HIV-unexposed children [6, 8–11], predominantly driven by increased frequency and severity of common childhood infections, particularly respiratory disease [6, 8, 9, 12–15]. Recent meta-analyses estimate twofold higher child mortality amongst HEU compared to HIV-unexposed children in the first 1–2 years after birth, with a similar risk persisting in children between 2 and 5 years of age [10, 11]. Finally, a clear divide in the categorisation of studies comes from the use of ART. Studies from the pre-ART era have the benefit of exploring the effect of HIV exposure without the potential confounding effects of ART. However, they may be less relevant in the modern era where most HEU children are also ART-exposed. Although there are clear benefits from ART in reducing HIV transmission and improving maternal health, there is the potential for ART to have negative effects on the developing fetus, and separating the effects of HIV and ART exposure is challenging.

Growth and Neurodevelopment of HEU Children

An estimated 250 million (43%) children under the age of 5 years fail to reach their developmental potential in low- and middle-income countries (LMIC), based on proxy measures of stunting and poverty [16••]. Impaired growth and development have far-reaching consequences across the life-course, impacting academic outcomes, employment and long-term non-communicable disease risk, as well as intergenerational effects on health and human capital (Fig. 1). Emerging data indicate that this cycle may be critically influenced by HIV exposure.

Growth Outcomes

Linear growth is an important reflection of overall child wellbeing. In the largest cohort to date, Zimbabwean HEU children in the pre-ART era had 23% more stunting (length-for-age Z score < -2) than HIV-unexposed children from the same communities by 12 months of age [17]. The effect of HIV exposure on growth in the ART era is less clear because there have been few studies to date. Reducing antenatal HIV exposure through ART control of maternal viraemia during pregnancy decreases the risk of transmission and may have additional benefits for HEU infants. However, intrauterine growth restriction and preterm birth have been associated with certain antiretroviral drugs in some studies [18•]. Findings from two large cohorts in the ART era have recently been reported in two different African settings. In Cape Town, where overall stunting prevalence was low, HEU children

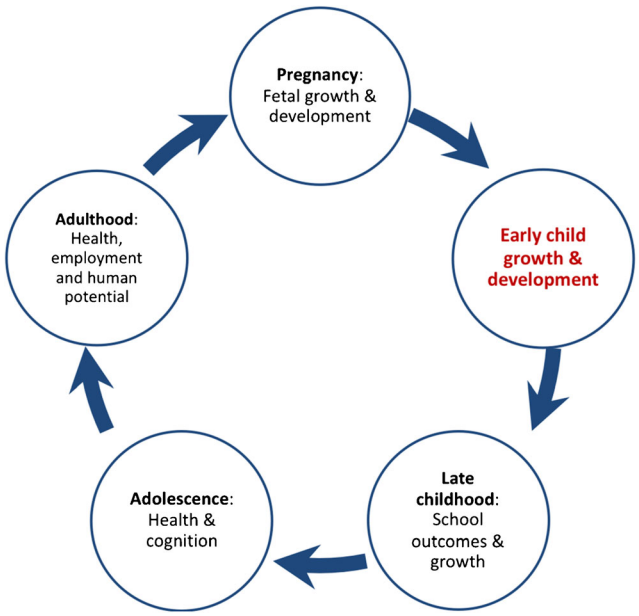

Fig. 1 The cycle of child growth and development

had lower length-for-age *Z* scores and almost threefold more stunting than HIV-unexposed children [19•]. In rural Zimbabwe, where overall stunting prevalence was high, HEU children also had lower length-for-age *Z* scores and almost twice as much stunting [20]. Similar findings were seen for weight-for-age, underweight (weight-for-age *Z* score < − 2), head circumference and microcephaly (head circumference-for-age *Z*- score < − 2), although not weight-for-length or wasting (weight-for-length *Z* score < − 2) [20]. Collectively, these studies indicate that growth impairment continues to be a major problem amongst HEU children in the current PMTCT era.

Neurodevelopmental Outcomes

The negative impact of HIV infection on child brain development, both clinically and neuroradiologically, is well-established [21, 22]. There is increasing evidence that HIV exposure (without infection) may also be associated with neurodevelopmental impairment, although the manifestations are more subtle than for HIV-infected children [21, 23, 24••]. Studies from LMIC settings report an impact of HIV exposure on language [21–23, 24••, 25, 26], behaviour [21, 23], cognition [24••, 26] and motor function [24••, 27], although there are limitations to these studies: most were from the pre-ART era, had small sample sizes and lacked adequate comparator groups. A large Zimbabwean study from the pre-ART era found that head circumference was consistently lower in HEU compared to HIV-unexposed infants throughout the first year after birth [28], but there were no neurodevelopmental evaluations in this cohort.

Recent studies in sub-Saharan Africa have generally supported findings from the pre-ART era. One study of South African HEU children aged 12 months found increased odds of cognitive (OR 2.28, 95% CI 1.13, 4.60) and motor delay (OR 2.10, 95% CI 1.03, 4.28) [29•], while another from Botswana showed increased expressive language delay (aOR 1.44, 95% CI 1.01, 2.06) at 2 years [30]. HEU children aged 2 years in rural Zimbabwe had poorer motor and language development compared to their HIV-unexposed community counterparts [31] and the Drakenstein Child Health Study found receptive and expressive language delay in South African HEU children compared to HIV-unexposed children at 2 years of age [32•].

In contrast, a recent study from Uganda and Malawi did not find any neurodevelopmental differences in HEU children aged 1 to 5 years compared to an HIV-unexposed group [33•]. Similarly, evidence from HEU children in high-income settings has generally been reassuring [34, 35]. However, some language delay has been reported [36, 37] and there is a suggestion that cognition and behaviour may be affected at older ages [21, 23] [38]. A UK study found similar outcomes for HIV-infected adolescents and their uninfected siblings, and that both groups were impaired compared to normative data [39]. There are few studies of older HEU children in LMIC settings from the current ART era. One multisite study across five African countries did not find any cognitive differences compared to HIV-unexposed children [40], although language skills were not reported. Other studies have indicated potential ongoing delays, including poorer school mathematics performance in Zambia [41], and lower IQ, language and fine motor development in HEU children aged 2–12 years in Thailand and Cambodia [26].

Overall, although further research is needed, current evidence suggests HEU children may be at risk of delayed neurodevelopment in the early years of life, particularly in LMIC settings, and there are concerns regarding later school and behavioural outcomes.

Pathogenesis—Universal Pathways Versus HIV-Specific Pathways?

We hypothesise that there are two broad pathways through which HIV exposure without infection may impact child growth and development: (1) indirectly, by augmenting existing universal pathways that are known common risk factors for poor growth and development; and (2) directly, via HIV-specific mechanisms including exposure to HIV virions, immune activation and ART toxicity **(**Fig. 2**)**.

Augmentation of Universal Pathways

Growth and development entail the interaction of genetically determined biological processes and environmental influences. The ecological model of development [42] describes the interacting nature of these internal and external factors over time. Both the cumulative burden and timing of these risks likely influence neurodevelopment [43]. Research suggests that HIV exposure may augment a range of universal risk factors, and below, we outline the available evidence.

Intrauterine Infections

Many in utero infections have consequences for the developing fetus. Cytomegalovirus (CMV), rubella, Zika virus, syphilis and toxoplasmosis have all been documented to impact child development [44]. HIV-infected mothers are at increased risk for some of these infections; several studies, although not all, have indicated that CMV in particular may be more prevalent in HIV-affected mothers and children [45•, 46] and may explain some of the adverse outcomes associated with HIV exposure [47].

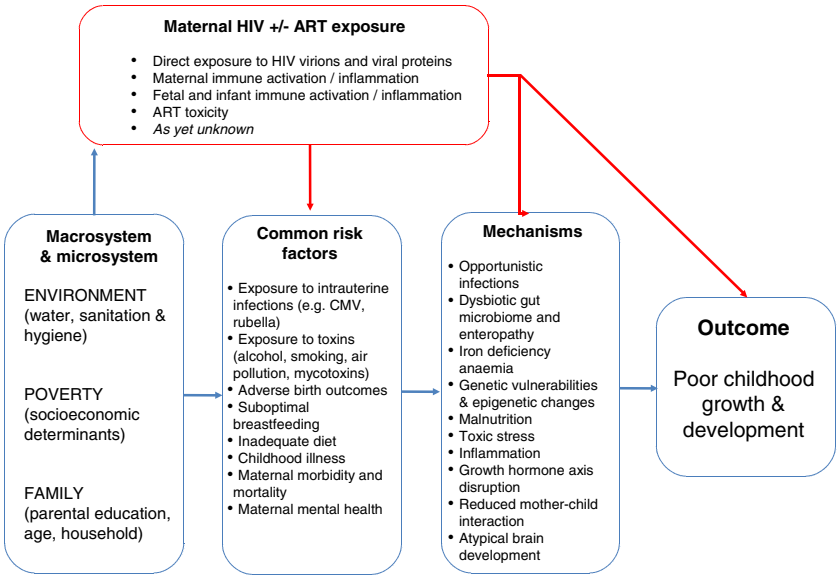

Fig. 2 Conceptual framework of the hypothesised pathways through which HIV and ART exposure affect child growth and development. Red lines demarcate HIV-specific pathways; blue lines represent universal pathways. ART, antiretroviral therapy

Toxins

Exposure to alcohol, tobacco and recreational drugs has a dose-related impact on child growth and development [48, 49]. This is potentially through direct toxicity, augmentation of inflammatory upregulation, as well as poorer maternal health-seeking and parenting behaviour. HIV infection in adults is associated with an accentuated risk of alcohol and substance use in many settings [34]. Air pollution and environmental toxins (such as mycotoxins) are also potential risk factors for poor child growth [50, 51]. There is some evidence that HIV-infected individuals may have higher mycotoxin levels despite the same exposure when compared to HIV-uninfected controls. This may be due to impaired liver function resulting in a decreased ability to detoxify metabolites, though not all reports have corroborated this finding [52, 53]. Mycotoxin exposure may plausibly exacerbate several pathways underlying HIV pathogenesis (including enteropathy and micronutrient deficiencies), thereby affecting early growth [54]. Additionally, the interacting effects of air pollutants and HIV have been associated with adverse birth outcomes, potentially through increasing nitric oxide levels [51].

Adverse Birth Outcomes

Prematurity and small-for-gestational age (SGA) are consistently associated with poor postnatal growth and development [55, 56]. Premature infants are vulnerable to prolonged hospitalisation and are at greater risk for neonatal infection and sepsis [57]. HIV-exposed children are at increased risk of prematurity, SGA and low birth weight [58], which are major risk factors for stunting [59]. Evidence suggests that HIV exposure remains associated with adverse birth outcomes and neonatal death despite maternal ART [60, 61]. Separately, ART exposure has been associated with adverse birth outcomes. A recent meta-analysis found that HIV-positive women who conceived on ART had 41% greater risk of preterm delivery compared to HIV-positive women who started ART during pregnancy [18••]. A study from Botswana showed this risk is similar between efavirenz- and dolutegravir-containing regimens [62]; however, recent concerns have been raised around the safety of dolutegravir at the time of conception due to a potential increase in neural tube defects [63]. Further data on the impact of specific drugs and the relationship between poor birth outcomes and timing of ART initiation are urgently needed.

Breastfeeding Practices

Global recommendations are that breastfeeding should be early (initiated within 1 h of birth), exclusive (breastmilk and prescribed medications only for the first 6 months of life) and prolonged (through 2 years of age). Suboptimal breastfeeding increases morbidity and mortality, particularly in LMIC settings [64]. A review of 17 observational studies demonstrated that better cognitive outcomes were associated with optimal breastfeeding practices [65]. Findings from a 2015 analysis of the 1982 Pelotas birth cohort in Brazil also reported a dose–response association between breastfeeding duration and increased child cognitive performance, educational attainment and income at the age of 30 years [66]. Globally, guidance on infant feeding for HIV-positive mothers in LMIC settings has changed over time with the introduction of PMTCT and recognition of the benefits of breastfeeding, despite the risk of postnatal HIV transmission [67]. However, formula feeding to prevent postnatal HIV transmission through breastmilk means that HEU children continue to have lower rates of breastfeeding across the world, which may

influence developmental outcomes. The impact of postnatal ART exposure through breastfeeding is still to be examined.

Inadequate Diet

Food insecurity in pregnancy is a risk factor for adverse birth outcomes and, during early childhood, has been associated with poor growth and neurodevelopment arising from complex mechanisms including nutritional insufficiencies and increased parental stress [68]. Children need sufficient nutrients for growth and immune development, and dietary diversity is necessary for optimal child outcomes. Food insecurity often coexists with HIV infection [69] and has been found to be associated with lower ART adherence, increased HIV-associated illness and decreased survival in HIV-infected individuals [70].

Childhood Illness

Illnesses such as childhood pneumonia are associated with long-term physical sequelae [71]. If hospitalisation is needed, this may also have considerable impact on children and their families [72], causing separation from parents and reducing school attendance. Mounting evidence suggests HEU children have increased infectious morbidity [6, 15, 73], and studies to date have found this is particularly due to viral and bacterial respiratory infections [14, 74, 75] in early life. There is also evidence that HEU children are at risk for unusual infections [76], including *Pneumocystis jirovecii* [9, 77–82], CMV [79, 83, 84] and haemorrhagic varicella [12, 77]. Correspondingly, cohort studies from both LMIC and high-income settings suggest HEU children have a higher risk of hospitalisation during infancy [85–87] as well as elevated mortality [10]. In pre-ART era Zimbabwe, morbidity and mortality of HEU children were strongly associated with maternal HIV disease severity, and increased infectious morbidity amongst HEU children remained until maternal CD4 counts were ≥ 800 cells/μL [88]. In the ART era, a recent study from Belgium found that initiation of maternal ART prior to pregnancy (i.e. conception occurred on ART) appeared protective against infectious morbidity in HEU children; however, further work is needed to explore these findings in LMIC [86].

Maternal Illness and Death

Maternal physical health before and after birth critically influences child growth and development. Mothers who are unwell may be less able to bond with and care for their children and maternal undernutrition is a risk factor for adverse birth outcomes [59, 89]. Furthermore, the loss of one or more parents, orphanhood and institutionalisation have severe repercussions [90]. There is extensive literature from the pre-ART era on the impact of parental death from HIV/AIDS, and it is estimated over 17 million children have lost one/both parents from the HIV/AIDS epidemic [90]. Parental death may affect children emotionally and impact subsequent relationships. Maternal death is itself related to outcomes of HEU infants [91], potentially for several reasons. First, an infant born to a mother with advanced disease may have greater immune abnormalities; second, mothers who are sick in the late stages of their illness may be unable to care for their children adequately, both physically and emotionally; and third, children who lose their mothers may be subject to extreme poverty and homelessness [92]. Even in the ART era, maternal HIV infection is associated with higher morbidity and mortality, and disease severity in HIV-infected mothers has been associated with infant health outcomes [93].

Maternal Mental Health

Maternal psychological illness is linked to adverse child health outcomes. Maternal depression is a risk factor for impaired child growth and development [94, 95]. Similarly, maternal stress has been found to have long-term repercussions on child psychological health [96]. In many communities, there is an association between maternal stress or depression and HIV [97], and the additional exposure to maternal mental health problems has been associated with risk of poor cognitive development in HEU children [98]. Maternal capabilities, which reflect the attributes required to care for a child, may be affected by HIV infection. HIV-infected parents who are unwell may be at risk of poverty because of an inability to work, which may affect availability of food and access to healthcare for the household. HEU children may be expected to take on caregiver roles for infected family members or may be neglected when other children in the family are HIV-infected [39]. Using models to approximate the effect of maternal HIV on young children in LMIC settings, it has been estimated that, in HIV-affected families, school completion falls from 61 to 57%, and children have a 10% higher incidence of anxiety or depression than HIV-unexposed children [99–102].

HIV-Specific Pathways

Children born to HIV-infected mothers may be exposed to both HIV and ART antenatally, perinatally and/or postnatally. It is likely that in addition to the universal pathways discussed above, there are separate HIV-related mechanisms that impact on the child's growth and development including (i) directly through exposure to HIV virions, (ii) through effects of maternal immune activation/inflammation on the in utero environment, (iii) by promoting immune activation/inflammation in the fetus and/or child and (iv) via ART toxicity throughout the period of in utero development and breastfeeding.

Direct Exposure to HIV Virions and Viral Proteins

HIV is a neurotropic virus, which can cause encephalopathy in HIV-infected infants, leading to microcephaly and neurodevelopmental impairment [21]. Neuropathology occurs directly, following exposure to HIV proteins that exhibit neurotoxicity, and indirectly via microglial activation and neuroinflammation. It is conceivable that antenatal exposure to HIV may affect brain development even without infection. Studies have detected HIV-specific immune responses in HEU children, suggesting that sufficient HIV antigen exposure occurs antenatally or perinatally to prime immune responses [103]. Direct exposure of the fetal brain to HIV virions and proteins may therefore be hypothesised to cause HIV-mediated neurotoxicity and impact brain growth and development. It is possible that in utero exposure to HIV virions may also modulate immune responses, resulting in adverse outcomes, similar to other chronic maternal infections including malaria, as discussed below [103].

Maternal Immune Activation/Inflammation

A healthy, regulated immune system is important for neurodevelopment [104]; therefore, HIV-related maternal immune activation and chronic systemic inflammation may impact fetal development. Advanced maternal HIV disease antenatally, which is driven by immune activation, has been consistently associated with HEU child morbidity [86]. Le Roux and colleagues recently found that the duration and severity of HIV viraemia in mothers during the antenatal period was closely related to developmental outcomes in their HEU infants, but immune activation was not assessed in this study [105]. Dysregulated immune mechanisms have been linked to various neurological disorders, and epidemiological associations exist between infections in pregnancy, maternal immune activation and schizophrenia, autism and epilepsy in offspring [106, 107], suggesting biological plausibility for this hypothesis in the setting of HIV. Further studies of HIV-positive mothers and HEU offspring are required in order to evaluate associations between inflammation in pregnancy and child development outcomes in these critical early years.

Immune Activation/Inflammation in the HIV-Exposed Uninfected Child

Immune activation and inflammation have been demonstrated in HIV-exposed fetuses and infants [108] which may trigger cytokine release, directly impacting cell migration and axonal growth and overall brain development [109–111, 112••]. Similarly, growth hormone axis disruption of Zimbabwean HEU children has been associated with systemic immune activation and the level of CMV replication [113]; inflammation in these infants was predominantly driven by higher HIV viral loads in mothers transmitting CMV [46]. Taken together, there appears to be a complex relationship between maternal HIV viraemia, early-life CMV acquisition, infant immune activation and the growth hormone axis, which may plausibly contribute to poor linear and brain growth in HEU children, and subsequent impaired neurodevelopment. Immune activation and inflammation may also drive neurodevelopmental impairment directly by impacting cell migration and axonal growth via proinflammatory cytokines [112••].

ART Toxicity

Antiretrovirals cross the placental barrier with varying concentrations, and their potential effects on offspring growth and development continue to be investigated [114]. It is challenging to disentangle the effects of HIV and ART, particularly because treatment improves maternal health, which may offset any negative impact. Although ART is clearly essential both for maternal health and to reduce vertical transmission of HIV, exposure has been associated with adverse outcomes in some studies [25], including prematurity, poor growth, metabolic disturbance [115–117] and mitochondrial abnormalities [118–120]. Other studies have found no serious adverse effects [121, 122]. The Pediatric HIV/AIDS Cohort study (PHACS) has established the Surveillance Monitoring for ART Toxicities in HEU children (SMARTT) study to monitor for ART toxicities across a range of metabolic, growth, cardiac and neurological outcomes [123, 124]. Results indicate little adverse effect of maternal ART on child outcomes [35]; however, atazanavir has been found to potentially impact language acquisition [25, 35, 36, 125], and tenofovir has been associated with an adverse impact on bone mineral content [126]. Recently, concerns have been raised over neural tube defects following dolutegravir exposure at conception [127•]. Children may additionally be exposed to ART via breast milk or directly as prophylaxis. Transient haematological alterations, including anaemia, have been associated with zidovudine exposure [128]. Further pharmacovigilance is needed to document the long-term safety of individual antiretroviral drugs and treatment combinations [108].

Mechanistic Pathways Mediating Impaired Growth and Development

Universal and HIV-specific risk factors may drive poor growth and neurodevelopment through a common set of mechanistic pathways, as outlined in Fig. 2, including co-infections, inflammation, enteropathy, anaemia, nutrient deficiencies, epigenetic modifications and toxic stress, ultimately impacting brain development. However, we lack data on many of these pathogenic processes, meaning studies are needed to further our understanding of the pathways within this conceptual framework. Here, we provide some insights that support these

mechanisms as potential mediators of the risk factors discussed above.

Direct exposure to opportunistic infections may cause neurotoxicity and impair growth. Congenital CMV infection in particular has been shown to have a profound impact on neurodevelopment, and the outcomes and mechanisms have been reviewed comprehensively elsewhere [129]. CMV acquisition in early life is very common in sub-Saharan Africa. The impact of CMV on growth and development may be even greater in HEU children and has recently been reviewed [45•]. Garcia-Knight and colleagues found that CMV viral load in early infancy was negatively associated with weight-for-age and head circumference-for-age *Z* scores in both HIV-exposed and HIV-unexposed children in rural Kenya [130], and Gompels and colleagues found that CMV was associated with growth and early child development in Zambia, particularly amongst those exposed to HIV [47].

Environmental enteric dysfunction (EED) is an almost ubiquitous subclinical disorder of the small intestine in LMIC. EED is characterised by villous atrophy, impaired gut barrier function, intestinal inflammation and microbial translocation, leading to systemic inflammation [131]. We have previously hypothesised that EED may be more severe amongst HEU compared to HIV-unexposed children [132], although a study of Zimbabwean infants at 6 weeks and 6 months of age showed similar levels of intestinal fatty acid binding protein (I-FABP), a marker of enterocyte damage, in HEU and HIV-unexposed infants. However, CRP was consistently higher in HEU compared to HIV-unexposed infants, highlighting that systemic inflammation is greater in the setting of HIV exposure, although the drivers of inflammation remain poorly defined [133]. Proinflammatory cytokines appear to be upregulated in HEU compared to HIV-unexposed children even at birth, and certain brain maturation processes such as cell migration and axonal growth may be particularly vulnerable to this inflammatory milieu [111].

A common co-morbidity with stunting is iron deficiency anaemia (IDA). IDA is a major cause of neurodevelopmental impairment [43] and has been identified as one of the leading causes of years lived with disability in children [134]. HIV-exposed children have a higher frequency of anaemia than HIV-unexposed children [135, 136], plausibly driven by exposure to the virus itself and/or exposure to ART [137]. HIV infection is associated with other micronutrient deficiencies [52], and similar effects may be seen in HEU children, although data are currently lacking.

Early-life programming may be influenced by the in utero environment, causing DNA methylation and gene expression modifications [138]. These epigenetic modifications are now understood to have potential biological impact across the lifespan, leading to the theory of the developmental origins of health and disease and may have intergenerational effects [139]. HIV infection has been shown to lead to epigenome-wide differential DNA methylation in infected individuals [140]. Additionally, some studies have indicated that maternal HIV infection is associated with epigenetic modifications in neonates, and HIV and ART-exposed children have been found to have reduced DNA methylation in peripheral blood repetitive elements which may have long-term implications [141]. Further work is needed to understand this area; however, research into HIV-associated neurocognitive disorders (HAND) in adults indicates a role for genetic and epigenetic profiles in predicting vulnerability to the neurological effects of the virus and ART side effects [142].

Children living in adverse environments are at risk of chronic stress and persistent activation of their physiological stress response—a process known as toxic stress [143]. This process may act via the hypothalamic–pituitary–adrenal (HPA) axis and immune responses to disrupt healthy brain circuit development, particularly in the prefrontal cortex [144]. Through this impact on the neuroendocrine–immune (NEI) network, early experiences can fundamentally shape the developing brain architecture [143]. Early mutual interactions and experiences between children and key adults also shape the developing brain architecture and affect the NEI network [143]. Maternal physical and psychological illness coupled with a lack of social support may impact on a mother's ability to provide a safe and secure physical and emotional environment for her infant [145]. Similarly, growing up in an environment with inadequate stimulation or few early learning opportunities is associated with reduced cognitive development [146]. Children growing up in families affected by HIV may face multiple adversities that affect the parent–child relationship including parental illness and death, mental illness, and stress from emotional, financial and social pressures [147].

Ultimately, the risk factors and mechanisms identified here may modulate brain growth and network development. In addition, there may be other risk factors and mechanisms, including those as yet unknown, influencing these children. The in utero period and early postnatal life is a time of substantial brain growth, when extensive neural network development and maturation take place [7]. The developing brain during this time is particularly sensitive to environmental influences [148]. Animal models as well as human studies suggest that infection-induced maternal immune activation impacts developing neural circuits [106]. Studies have indicated that maternal immune activation and induction of proinflammatory cytokines affecting the microbiota–gut–brain axis or eliciting the stress response through the HPA axis result in atypical brain development of the fetus [107, 149, 150]. Neuroimaging study findings of HEU-associated white matter microstructural changes early in life [110, 151]

suggest abnormal brain development as a potential mechanism for impaired child outcomes.

Improving HEU Child Growth and Developmental Outcomes

Healthy birth, growth and development form the foundations for later school performance, employment opportunities and long-term human capital and health. First and foremost, the continued global focus on reducing the burden of antenatal HIV infection is the key to eliminating HIV exposure in children. However, given the expanding population of HEU children, efforts that focus on prevention and intervention strategies to reduce the burden of poor HEU child outcomes are also needed [108].

Prevention

In order to develop successful prevention strategies, further research is needed to understand the effects of HIV exposure on child outcomes. Improved ART coverage in recent years means that family and home environments may be different to previous decades; infant feeding advice has shifted over time and the impact of breastfeeding patterns in this population is still being unravelled; children are now more commonly exposed to ART in utero and postnatally, and more women are conceiving on ART. Studies examining the relative contributions of HIV-related pathways to growth and developmental impairment in the context of universal risk factors in the era of ART are needed to inform and focus these strategies [93]. In order to detect early adverse outcomes and intervene, pharmacovigilance systems are needed to assess effects of ART on the growing and developing child. This is particularly relevant with the introduction of new drugs and regimens, and new approaches to prevent HIV infection in pregnant and breastfeeding women using pre-exposure prophylaxis (PrEP). The concerns raised over dolutegravir safety at the time of conception provide lessons going forward [63]. Evaluating the relative safety of different ART regimens in pregnancy for HEU child growth, brain health and development is critical.

Some studies suggest there may be a high-risk subgroup within the HEU population vulnerable to adverse outcomes. Studies have found interactions between HIV exposure and preterm birth leading to poorer growth [19•] and worse cognitive and motor outcomes [29•], and also poorer outcomes in HEU children with CMV coinfection, compared to those without [47]. Further evidence is needed to define these high-risk subgroups, who may benefit from targeted prevention strategies, particularly where resources are limited. Initiating prevention before conception to optimise the health of mothers and their families will likely be most effective.

Intervention

Alongside the development of prevention policies, intervention strategies are needed to identify and support HEU children at risk of growth and developmental impairment to improve long-term outcomes. Evidence from institutionalised children suggests interventions need to be initiated early (before the age of 5 years) to reverse impairment during critical periods of growth and development; however, the optimal window of opportunity for HEU children remains unclear [43].

Targeting the universal risk factors that impact growth and development is a logical strategy, through improved breastfeeding, adequate nutrition, support for maternal physical and psychosocial health, reducing exposure to toxins, prevention and early treatment of childhood infections and reducing poverty. Integrating this with improved maternal ART adherence throughout the period of pregnancy and breastfeeding to ensure HIV viral suppression would also likely contribute to improved outcomes amongst HEU children. This may be delivered as integrated care through multisectoral platforms targeting health, nutrition and education, involving both the caregiver and the child to break the intergenerational cycle, using approaches such as the UNICEF Nurturing Care Framework.

Concurrently, research informing additional targeted interventions to address the HIV-related pathways by assessing scalable packages of care for early child growth and development are needed. Recently, the SHINE trial in rural Zimbabwe demonstrated that the combination of improved infant and young child feeding and improved water, sanitation and hygiene interventions resulted in better outcomes in both motor and language development amongst HEU children [152]. Further work is required to examine the most effective, scalable intervention strategies moving forward.

Conclusions

There are currently 14.8 million HEU children worldwide and this population continues to expand. Increasing evidence suggests these children, particularly in LMIC settings, have a disparity in growth and development compared to HIV-unexposed children. Our conceptual framework highlights potential pathways linking HIV and/or ART exposure and adverse outcomes. We propose HEU children may be affected by accentuating existing universal risk factor pathways as well as through HIV-specific pathways, via final common pathogenic mechanisms. Overall, a multifactorial causal pathway is most likely to shape the growth and neurodevelopment of the

HEU child. More research is critically needed in the era of universal ART, to understand these pathways further and inform prevention and intervention strategies.

Compliance with Ethical Standards

Conflict of Interest Catherine J. Wedderburn, Ceri Evans, Shunmay Yeung, Diana M. Gibb, Kirsten A. Donald and Andrew J. Prendergast declare no conflicts of interest. Catherine J. Wedderburn, Ceri Evans and Andrew J. Prendergast are supported by the Wellcome Trust (203525/Z/16/Z; 203905/Z/16/Z; 108065/Z/15/Z).

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- •• Of major importance

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6

Chapter 6: Early Neurodevelopment of HIV-Exposed Uninfected Children in the Era of Antiretroviral Therapy: a Systematic Review and Meta-analysis

(Review paper)

Early neurodevelopment of HIV-exposed uninfected children in the era of antiretroviral therapy: A systematic review and meta-analysis

Summary

Chapter 6 presents the third research paper titled 'Early neurodevelopment of HIV-exposed uninfected children in the era of antiretroviral therapy: A systematic review and meta-analysis'. This summarises the research to date on the early neurodevelopment of children who are HEU in the current ART era, placing the results of Chapter 4 in the context of the wider literature, addressing objective 1. Through examining head circumference and neuroimaging, this chapter sets the scene for objectives 2 and 3. Evidence for the effect of ART on neurodevelopment is also considered (secondary objective).

Over half of the studies included in the systematic review reported poorer neurodevelopment in HEU compared to HU children, although findings were heterogeneous and methodological quality varied. The meta-analysis of ~5000 children from eight large high-quality studies, seven from Africa, found that HEU children are at risk of subtle impairments in expressive language and gross motor development by age two years compared to HU children. Although the effect sizes are small, given the numbers of HEU children worldwide, even small proportions of developmental delay may have substantial implications for individual academic achievement and human capital. This builds on the language outcomes reported in Chapter 4. There was no consistent effect of the maternal ART regimens analysed, although evidence was scarce.

Although findings for head circumference were mixed, larger studies suggest a small reduction in head circumference in HEU compared to HU children. Only one small neuroimaging study was identified fitting the search criteria (children aged 0-5 years born after Jan 1, 2000). This was from the DCHS and reported white matter alterations associated with adverse neurodevelopment in neonates, paving the way for further work on potential neurological pathways. Overall, this paper highlights the need for large high-quality longitudinal studies and neuroimaging data to assess the neurodevelopmental trajectories of HEU children.

My role involved conception and design of the systematic review, which was registered on Open Science Framework (https://osf.io/yeqj3/); screening the literature with Ella Weldon (MSc student); assessing risk of bias with Cesc Bertran-Cobo (MSc student); extracting and summarising outcomes through narrative review; conducting the meta-analysis with advice

from A/Prof Andrea Rehman (statistician); interpreting results with input from Prof Kirsty Donald (supervisor) and Prof Andrew Prendergast (field expert); writing the first draft of the manuscript, and incorporating co-author edits and peer-review feedback.

Note: This paper contains study results from Chapter 4.

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Supplementary material

The supplementary material as detailed in the published article is available at https://doi.org/10.1016/S2352-4642(22)00071-2 and listed in Appendix VI.

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Student ID Number	383999	**Title**	Dr
First Name(s)	Catherine		
Surname/Family Name	Scrymgeour-Wedderburn		
Thesis Title	Neurodevelopmental effects of HIV exposure: a prospective neuroimaging study of uninfected children born to mothers living with HIV		
Primary Supervisor	Professor Shunmay Yeung		

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Early neurodevelopment of HIV-exposed uninfected children in the era of antiretroviral therapy: a systematic review and meta-analysis

Catherine J Wedderburn, Ella Weldon, Cesc Bertran-Cobo, Andrea M Rehman, Dan J Stein, Diana M Gibb, Shunmay Yeung, Andrew J Prendergast, Kirsten A Donald**

Summary

Background There are 15·4 million children who are HIV-exposed and uninfected worldwide. Early child development crucially influences later academic and socioeconomic factors. However, the neurodevelopmental outcomes of HIV-exposed uninfected (HEU) children in the era of maternal antiretroviral therapy (ART) remain unclear. We aimed to examine the effects of in-utero exposure to HIV and ART on child neurodevelopment.

Methods For this systematic review and meta-analysis, we searched MEDLINE, Embase, PubMed, Africa-Wide Information, PsycInfo, and Global Health databases from inception to May 27, 2020, for studies from the past two decades reporting neurodevelopment of HEU children aged 0–5 years compared with HIV-unexposed (HU) children (aim 1), and effects of different maternal ART regimens on neurodevelopment of HEU children (aim 2). We did narrative syntheses for both aims, and a random-effects meta-analysis of high-quality studies comparing HEU children and HU children, to obtain weighted pooled estimates of effect sizes. This study was registered with PROSPERO, CRD42018075910.

Findings We screened 35 527 records and included 45 articles from 31 studies. Overall, 12 (57%) of 21 studies comparing HEU children and HU children found worse neurodevelopment in HEU children in at least one domain. Study design and methodological quality were variable, with heterogeneity across populations. Meta-analysis included eight high-quality studies comparing 1856 HEU children with 3067 HU children at ages 12–24 months; among HEU children with available data, 1709 (99%) of 1732 were exposed to ART. HEU children had poorer expressive language (effect size –0·17 [95% CI –0·27 to –0·07], p=0·0013) and gross motor function (–0·13 [–0·20 to –0·07], p<0·0001) than HU children, but similar cognitive development (–0·06 [–0·19 to 0·06], p=0·34), receptive language development (–0·10 [–0·23 to 0·03], p=0·14), and fine motor skills (–0·05 [–0·15 to 0·06], p=0·36). Results suggested little or no evidence of an effect of specific maternal ART regimens on neurodevelopment; study heterogeneity prevented meta-analysis.

Interpretation HEU children are at risk of subtle impairments in expressive language and gross motor development by age 2 years. We found no consistent effect of maternal ART regimens analysed, although evidence was scarce. We highlight the need for large high-quality longitudinal studies to assess the neurodevelopmental trajectories of HEU children and to investigate underlying mechanisms to inform intervention strategies.

Funding Wellcome Trust and Medical Research Council.

Introduction

Widespread access to antiretroviral therapy (ART) in pregnancy has substantially reduced vertical HIV transmission, meaning most children born to mothers with HIV are HIV-exposed and uninfected. There are an estimated 15·4 million HIV-exposed uninfected (HEU) children worldwide, comprising over 20% of annual births in some high HIV-burden countries. Disparities in early-life mortality and morbidity are evident between HEU children and HIV-unexposed (HU) children, and concerns have been raised regarding the effects of HIV and ART exposure on neurodevelopment.[1,2]

Early child development forms the basis of future academic achievement and socioeconomic outcomes.[3] The Sustainable Development Goals recognise the importance of child neurodevelopment, since the early years are foundational for brain development.[4] It is important to understand the manifold risk factors for impaired development to inform intervention strategies that enhance child neurodevelopment potential. However, neurodevelopment is difficult to measure at young ages, making it challenging to interpret findings from single studies.

Existing literature suggests that HEU children might be at risk for adverse cognitive, language, and motor outcomes compared with HU children. However, previous systematic reviews describing neurodevelopmental delay include multiple reports from before widespread access to ART and few studies from countries with generalised HIV epidemics.[5–7] Although two meta-analyses have been done, both were restricted to studies that used the Bayley

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For more on **HIV and AIDS** see https://aidsinfo.unaids.org/

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Research in context

Evidence before this study

Children who are HIV-exposed and uninfected represent a growing global population. The neurodevelopmental outcomes of this group of children in the era of antiretroviral therapy (ART) remain unclear; however, evidence is emerging from recent studies. Further information is needed to understand the nature of early neurodevelopment in this population to inform provision of care to improve outcomes. On searching the literature, we identified two meta-analyses but these were limited by the low number of included studies, including some with high risk of bias, thereby leaving a gap in our understanding of the neurodevelopmental outcomes of HIV-exposed uninfected (HEU) children in regions of high HIV prevalence. Individual studies were often limited by sample size and although previous reviews have been done, the majority have included studies from before widespread access to ART or have few studies from sub-Saharan Africa where the highest burden of HIV exists. We are unaware of any systematic reviews that have assessed the effect of different maternal ART regimens or examined imaging and head circumference alongside neurodevelopmental assessments. There is a need for an updated and focused synthesis of data for HIV and ART exposure on neurodevelopment in the ART era.

Added value of this study

Our systematic review included 45 articles (from 31 studies) building on previous reports by contributing updated data following widespread access to ART. Our meta-analysis combined larger, high quality studies, mostly from sub-Saharan Africa, reflective of the current population of HEU children. We found that HEU children have worse expressive language and gross motor development compared with HIV-unexposed children with small effect sizes, but with similar cognitive, receptive language, and fine motor skills. To our knowledge, this is the first systematic review to assess effects of different maternal ART regimens on the neurodevelopment of HEU children. We found that few studies explored the effects of ART, but the scarce evidence suggests that there is little, if any, effect of the specific ART regimens or drug classes assessed on neurodevelopmental outcomes, although concerns were raised for efavirenz and atazanavir.

Implications of all the available evidence

HEU children are at risk for subtle impairments in expressive language and gross motor development in early life. Although effect sizes were relatively small, the large number of HEU children worldwide means that even these subtle deficits might have a substantial effect in high-HIV burden countries, particularly in environments with multiple overlapping risk factors. Supporting these children to thrive might require interventions that focus on expressive language and gross motor skills in early childhood. Future research with large, high-quality, longitudinal studies is needed to examine outcomes at older ages and to investigate underlying mechanisms, with consistent methodology and standardised tools across settings to inform prevention and intervention strategies.

Scales of Infant and Toddler Development, some with high risk of bias.[7,8] Further, in one meta-analysis, all studies from outside the USA were classified as low quality because of potential confounding and small sample sizes,[7] thereby limiting understanding of outcomes of HEU children in regions of high HIV prevalence.

Access to triple-drug ART during pregnancy and breastfeeding has expanded, leading to improved maternal survival and higher breastfeeding rates, which might influence neurodevelopment.[9] Several studies from after the rollout of ART have reported that HEU children remain at risk of delayed neurodevelopment;[10,11] however, other studies found no differences when compared with HU children.[12,13] Separately, in-utero exposure to ART has been associated with adverse neurodevelopment.[14] Due to heterogeneity across studies and populations, including differences in maternal ART use and neurodevelopmental assessment tools, uncertainty remains regarding the outcomes of HEU children in the present day. Our first aim was to examine the effect of in-utero HIV exposure on child neurodevelopment through a comparison of HEU children and HU children, and our second aim was to investigate the effect of in-utero ART exposure on the neurodevelopment of HEU children.

See Online for appendix

Methods

Search strategy and selection criteria

We searched MEDLINE, Pubmed, Embase, PsychINFO, Global Health, and Africa-Wide Information without language restrictions from database inception to May 27, 2020. We used search terms for "child", "neurodevelopment", and "HIV/ART", which were adapted for each database. MeSH headings were also used in MEDLINE, and Emtree terms in Embase, combined with database-specific filters. The search strategy and search terms are in the appendix (pp 3–6). The reference lists and citations of eligible papers were searched for additional studies.

We defined our inclusion and exclusion criteria in line with the Population, Exposure, Comparator, Outcomes[15,16] framework (appendix p 7). Eligible studies included HEU children aged 0–5 years born after Jan 1, 2000. We excluded studies in which antiretroviral drugs were unavailable at the time. For our first aim,

we examined in-utero exposure to HIV and included studies comparing HEU children with HU children. For our second aim, we investigated exposure to maternal ART (defined as at least one antiretroviral drug in pregnancy) and included studies comparing HEU children exposed to different ART regimens, classes, or drugs, or no treatment; we did not require these studies to have a comparison group of HU children. The coprimary outcomes were cognitive development, receptive language, expressive language, fine motor and gross motor development, and social-emotional and adaptive behaviour. Secondary outcomes were head circumference and brain structure. All study designs (interventional, and observational cohort, longitudinal, and cross-sectional) in English or Spanish were included. We excluded conference and poster abstracts.

EW and CJW independently screened all titles, abstracts, and full texts for eligibility. Differences were resolved by discussion with a third reviewer (KAD or AJP). Where relevant, authors were contacted to clarify study eligibility. Search results were de-duplicated in EndNote X8. Study quality and risk of bias of studies comparing neurodevelopment of HEU children and HU children were assessed independently by two authors (CB-C and CJW) using the validated National Heart, Lung, and Blood Institute's Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies.[17] We adapted the assessment similarly to a previous review[18] (appendix p 8). Studies were given an overall quality rating of good, fair, or poor as recommended by COSMOS guidance,[15] based on low, medium, or high risk of bias, respectively.

This systematic review and meta-analysis followed the PRISMA guidelines.[19] The protocol was registered on PROSPERO (CRD42018075910; appendix p 2).

Data extraction

Data extraction was done in duplicate (by CJW, EW, and CB-C), including study design and setting, population demographics, exposure (HIV testing and ART exposure), methodology, outcome measures, and results. Outcomes of HEU children were classified by comparison with HU children as better, worse, or no difference on the basis of the individual study significance testing ($p<0·05$) or absolute or relative differences with confidence intervals in cases where p values were not shown.[20] Studies comparing different maternal ART regimens were classified with the same method, with one of the ART regimens selected as the reference group. For papers in the meta-analysis, aggregate mean scores with SDs of each neurodevelopmental domain for HEU children and HU children groups were extracted from individual studies. Where mean scores and SDs were not given or the neurodevelopmental outcomes did not fall within the specified domain groupings, we contacted authors for further information.

Data analysis

Our first aim was to investigate the effect of intrauterine HIV exposure. We did a narrative synthesis of coprimary outcomes compared between HEU children and HU children. We assessed unadjusted results, since studies adjusted for different confounders, and noted any changes on adjusted analyses where reported. For each study, significant differences in neurodevelopmental scores or proportions of developmental delay between the two groups in each domain were recorded and presented using a similar approach to Prado and colleagues.[21]

We did a meta-analysis of outcomes reported by six or more studies. As we anticipated substantial methodological heterogeneity and potential for confounding when assessing neurodevelopment, we limited this to high-quality studies and used a random-effects model. Due to the relatively wide age range in some studies, we used age-standardised values, where reported, and did sensitivity analyses using raw scores given the concern that the norms might overestimate development.[22] Given assessment tools differed across studies, we calculated weighted effect sizes (standardised mean differences with Hedge's correction; mean of HEU children minus mean of HU children, then divided by the pooled SD) with 95% CIs using the group mean and SD for each neurodevelopmental domain. Heterogeneity was estimated with the I^2 test, and Q-values were used to test for between-group differences. We planned to construct a funnel plot to examine publication biases where there were ten or more studies. Statistical significance was set at $p<0·05$.

For our second aim, we examined associations between maternal ART exposures, stratified by regimen, drug class and individual drugs (where available), and neurodevelopmental domains. We report a synthesis of results in a similar format to the primary aim. If ART studies were sufficiently similar, we planned a second meta-analysis. Finally, we reported a narrative synthesis of the effects of HIV and ART on head circumference and neuroimaging. We used Stata 16.1 for analyses and to derive forest plots.

Role of the funding source

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

A total of 35 527 references were screened (figure 1). We assessed 272 full-text studies, of which 227 did not meet inclusion criteria, most commonly due to failure to classify maternal and child HIV infection status (appendix pp 9–17). A total of 45 records reporting 31 studies were included, 44 in English and one in Spanish. Of these, 24 articles (21 studies) compared neurodevelopment between HEU children and HU children, 13 articles (ten studies) compared different maternal ART regimens, and

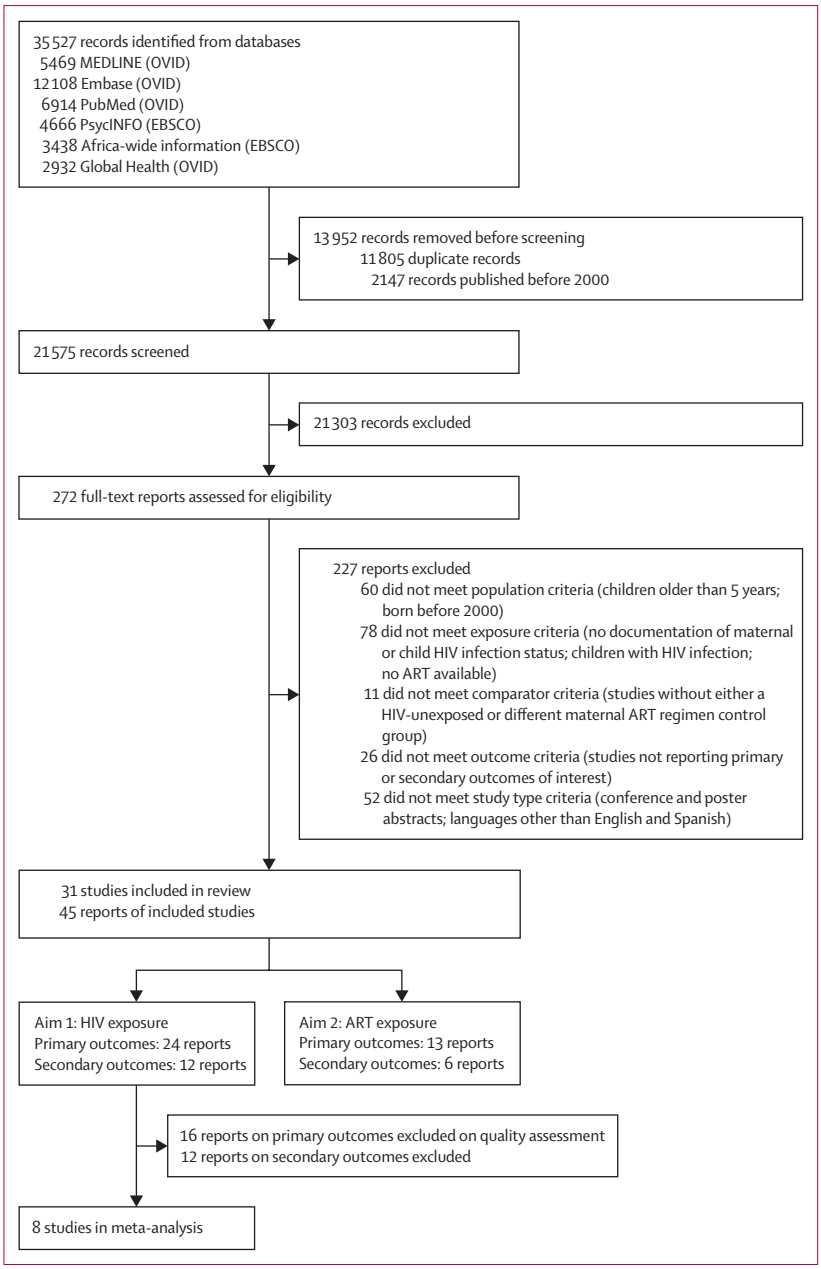

Figure 1: **Study selection**
At the record screening stage, the main categories for excluding records were: (1) population: age range over 5 years; (2) exposure: not examining HIV-exposed uninfected children or only reporting on children living with HIV; and (3) outcome: no neurodevelopment outcomes. Of the total number of reports included (n=45), five contributed results for the primary and secondary outcomes of the first aim, one contributed results for the primary and secondary outcomes of the second aim, and four contributed results for the primary outcomes of both aims (appendix p 18). ART=antiretroviral therapy.

18 articles (13 studies) reported head circumference or neuroimaging (figure 1; appendix p 18).

For the first aim, comparing neurodevelopment of HEU children versus HU children, 19 (79%) of 24 articles were from Africa,[10–13,23–37] two (8%) from South America,[38,39] two (8%) from North America,[14,40] and one (4%) from Asia.[41] Characteristics of the studies are detailed in figure 2 and the appendix (pp 19–20). The Bayley Scales of Infant and Toddler Development was the most common measurement tool (11 studies), followed by the Mullen Scales of Early Learning (four studies). All tools are listed in the appendix (p 21). In 12 (57%) of 21 studies, most HEU children were exposed to triple maternal ART (defined as at least three antiretroviral drugs during pregnancy).

Methodological quality varied, with eight reports judged good quality, 11 fair, and five poor (appendix p 22). Sample sizes ranged from 37 to 1380, with only 13 reports including over 50 children per group. The most common methodological concerns were selection bias and loss to follow-up. Blinded outcome assessments were described in eight (33%) of 24 reports. Of the 12 (50%) of 24 reports with adjusted analyses, covariates varied widely (appendix pp 19–20). The high-quality reports had representative study populations, included controls from the same community, and used validated outcome assessments.

Figure 2 shows the synthesis of results from all 24 reports. Two studies from South Africa[13,29–31] and one from Democratic Republic of the Congo[24,25] provided two reports each. The results were similar at each time-point in all three studies; therefore, our summary statistics only include each study once. Overall, 12 (57%) of 21 studies reported poorer neurodevelopmental outcomes in HEU children than in HU children in at least one domain on unadjusted analyses, eight (38%) reported no differences in neurodevelopment, and one (5%) found only better social-emotional development in HEU children versus HU children. In the 12 studies reporting adjusted analyses, most results remained unchanged; however, in one Canadian study reporting lower neurodevelopmental scores among HEU children, findings were attenuated after accounting for maternal substance use.[40]

Among the eight studies[10–14,23,32,33] eligible for meta-analysis, a slightly higher proportion of studies (five [63%] of eight) reported poorer outcomes across neurodevelopmental domains in HEU children. A total of 1856 HEU children and 3067 HU children from studies in Uganda (n=1), South Africa (n=3), USA and Puerto Rico (n=1), Botswana (n=1), Zimbabwe (n=1), and Malawi plus Uganda (n=1) were included. In all studies except one,[23] most mothers were taking triple ART during pregnancy; of the HEU children with available data, 1709 (99%) of 1732 had known ART exposure. Among children with regimen data, 1241 (75%) of 1661 were exposed to triple therapy and 414 (25%) of 1661 to zidovudine monotherapy. Forest plots are shown separately for the five neurodevelopmental domains reported in sufficient studies in figure 3. Since most studies reported outcomes at either age 12 or 24 months, we combined these and then did stratified sensitivity analyses. Only Boivin and colleagues[12] had observations at older ages; however, for consistency we only included their 24-month data.

	Country (time period)	HEU*	HU*	Age at assessment	Measurement tool†	Unadjusted analysis outcomes‡					Adjusted analysis
						CD	LD	MD	AB	SEB	
Kandawasvika et al (2011)[37]	Zimbabwe (2002–04)	188	287	3, 6, 9, and 12 months	BINS						..
Familiar et al (2018)[33]	Uganda (2012–15)	75	140	6 months	MSEL						Cognitive and gross motor results held in linear mixed effects models
				12 months	MSEL						
Le Roux et al (2018)[10]	South Africa (2013–16)	215	306	12 months§	BSID-III†¶						Cognitive results held; motor results attenuated
Laughton et al (2012)[30]	South Africa (2005–06)	28	34	12 months§	GMDS						..
Sirois et al (2013)[14]	USA and Puerto Rico (2007–11)	374	49	12 months§	BSID-III						..
Springer et al (2018)[13]	South Africa (2012–13)	58	38	12 months§	BSID-III & ADBB						..
Springer et al (2012)[28]	South Africa (2009)	17	20	17–19 months	GMDS						..
da Silva et al (2017)[38]	Brazil (not reported)	40	40	4, 8, 12, and 18 months (10 children per age)	BSID-III						..
Struyf et al (2019)[27]	Malawi (2008–11)	289	170	15 weeks, 6, 9, 12, 15, 18, and 24 months	BSID-III						..
Gomez et al (2009)[39]	Colombia (not reported)	23	20	3, 6, 9, 12, 18 and 24 months	BSID-II†‖						..
					DDST-II‖						
Landes et al (2012)[26]	Malawi (2008)	128	200	20 months	WHO milestones chart						..
Chaudhury et al (2017)[23]	Botswana (2010–12)	313	357	24 months	BSID-III						Cognitive results held; expressive language mean differences attenuated**
		337	386		DMC						HEU children scored higher in the personal-social domain
Ntozini et al (2020)[11]	Zimbabwe (2016–17)	205	1175	24 months	MDAT						Results held; in restricted analyses of the standard of care group (n=63; 373):
					CDI: vocabulary						MDAT gross motor and CDI vocabulary differences remained significant
					A-not-B task						
Wedderburn et al (2019)[32]	South Africa (2012–15)	61	199	6 months	BSID-III						Results held
		168	564	24 months	BSID-III¶						
Boivin et al (2016)[34]	Uganda (2010–13)	143	325	24 months	MSEL						Only lower receptive language was irrespective of other covariates examined
		122	331	36 months	MSEL						

(Figure 2 continues on next page)

	Country (time period)	HEU*	HU*	Age at assessment	Measurement tool†	Unadjusted analysis outcomes‡					Adjusted analysis
						CD	LD	MD	AB	SEB	
Springer et al (2020)[29]	South Africa (2012–13)	32	27	30–42 months	BSID-III						Results held
					SDQ						
Ngoma et al (2014)[36]	Zambia (2011–13)	97	103	15–36 months	FSDQ						Results held
Alimenti et al (2006)[40]	Canada (2003–04)	39	24	18–36 months	BSID-II†						Results lost significance on all domains
					VABS						
Wu et al (2019)[41]	China (2010–13)	250	250	6–36 months	BSID-III†						Cognitive results held; adaptive behaviour attenuated
Boivin et al (2019)[12]	Malawi and Uganda (2013–14)	405	456	12, 24,and 48 months	MSEL						Overall results held on longitudinal adjusted analyses
				48 and 60 months	KABC-II						
Laughton et al (2018)[31]	South Africa (2005–13)	34	39	11, 20, 30, 42, and 60 months	GMDS & Beery-VMI						..
Brahmbhatt et al (2014)[35]	Uganda (not reported)	105	108	0–72 months††	MSEL						HEU children had higher risk of cognitive and receptive language impairment
Van Rie et al (2008)[24]	Democratic Republic of Congo (2004–05)	13	20	18–29 months	BSID-II†¶						..
		19	31	18–36 months	RITL¶						
		22	70	30–72 months††	PDMS/ SONR¶						
Van Rie et al (2009)[25]	Democratic Republic of Congo (2004–05)	35	90	18–71 months††	BSID-II†/ PDMS/SONR						..

Cognitive development
Expressive language
Receptive language
Fine motor skills
Gross motor skills
Adaptive behaviour
Social-emotional behaviour
No difference
HEU children performed worse than HU children
HEU children performed better than HU children

Figure 2: **Neurodevelopment of HEU children compared with HU children**
CD=cognitive development. LD=language development. MD=motor development. AB=adaptive behaviour. SEB=social-emotional behaviour. HEU=HIV-exposed uninfected. HU=HIV-unexposed. ADBB=Alarm Distress Baby Scale. BINS=Bayley Infant Neurodevelopmental Screener. BSID-II=Bayley Scales of Infant and Toddler Development 2nd edition. BSID-III=Bayley Scales of Infant and Toddler Development 3rd edition. CDI=MacArthur-Bates Communicative Development Inventories. DDST-II=Denver Developmental Screening Test 2nd edition. DMC=Developmental Milestones Checklist. FSDQ=Full-Scale Developmental Quotient. GMDS=Griffiths Mental Development Scales. KABC-II=Kaufman Assessment Battery for Children 2nd edition. MDAT=Malawi Developmental Assessment Tool. MSEL=Mullen Scales of Early Learning. PDMS=Peabody Developmental Motor Scales. RITLS=Rossetti Infant-Toddler Language Scale. SDQ=Strengths and Difficulties Questionnaire. SONR=Snijders-Oomen Nonverbal Intelligence Test. VABS=Vineland Adaptive Behaviour Scales. Beery-VMI=Beery Buktenica Test of Visual Motor Integration. *Where the number differed across domains, the highest number is listed. †Where BSID-III composite scores are reported for language and motor development or BSID-II mental development index was used to reflect cognitive and language development; separately, where applicable, cognitive development was assessed using the MSEL cognitive composite score, MDAT total score, or GMDS general quotient. ‡Unadjusted analysis outcomes defined by statistical significance of p<0·05 or through 95% CIs in group comparisons of the mean or comparison of delay where applicable. §Age is given as 12 months if median age of assessment fell within 1 month of this time-point (appendix pp 19–20). ¶Delay reported here; on analysis of mean scores, Le Roux and colleagues[10] reported no significant group differences in mean scores; Wedderburn and colleagues[32] reported HEU children had lower receptive and expressive language scores than HU children in both unadjusted and adjusted analyses, and lower cognitive scores on unadjusted analysis. ||BSID-II differences at 6 and 18 months only, DDST differences at 6 months. **On analysis of adverse outcomes, HEU children had significantly more expressive language adverse outcomes than HU children on unadjusted and adjusted analyses. ††Studies included as median age within age range.

Overall, HEU children had worse expressive language outcomes (effect size –0·17 [95% CI –0·27 to –0·07], p=0·0013) and gross motor outcomes (–0·13 [–0·20 to –0·07], p<0·0001) compared with HU children, but similar cognitive development (–0·06 [–0·19, 0·06], p=0·34), receptive language development (–0·10 [–0·23

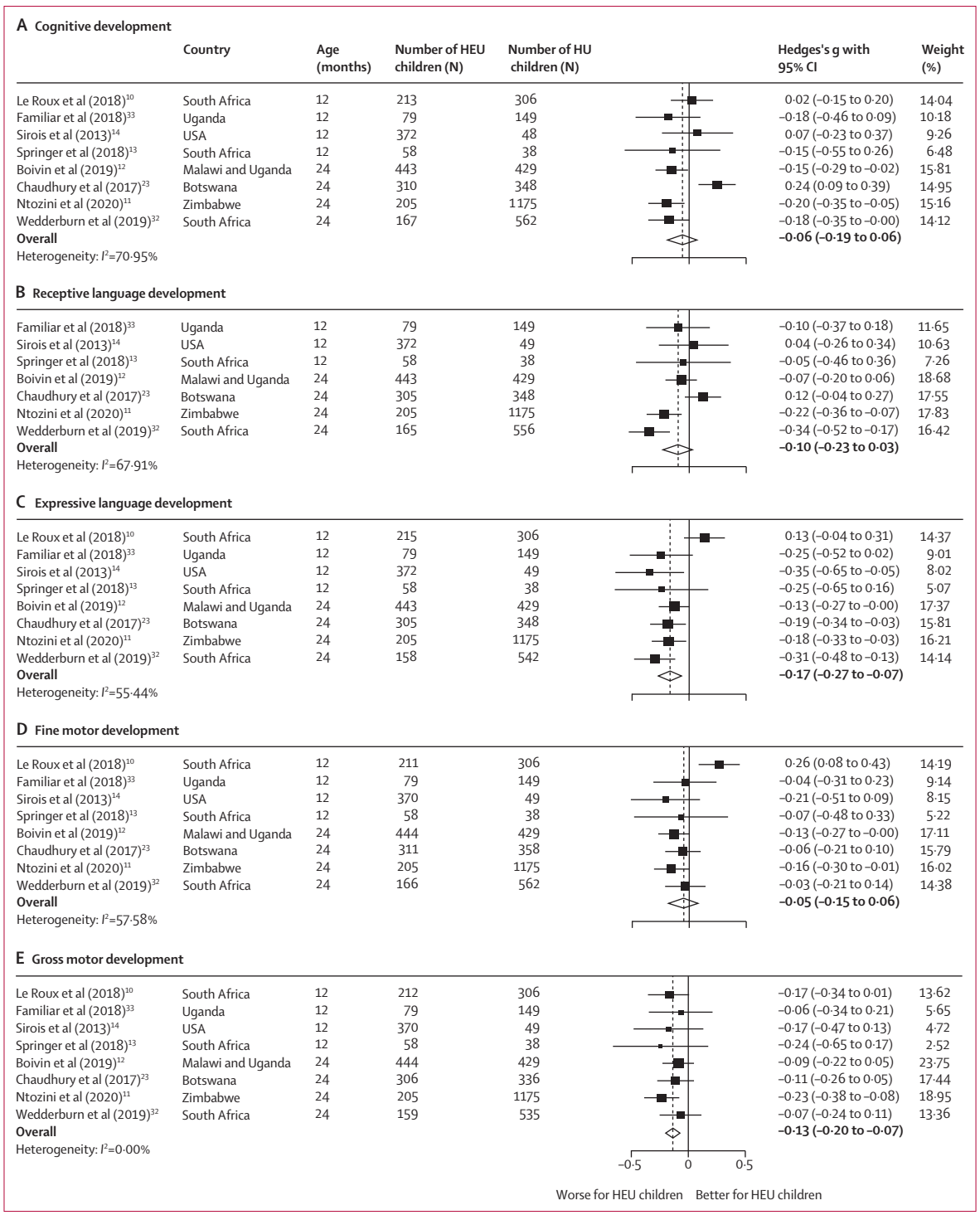

Figure 3: **Forest plots of neurodevelopmental outcomes of HEU children compared with HU children included in the meta-analysis**
HEU=HIV-exposed uninfected. HU=HIV-unexposed.

to 0·03], p=0·14), and fine motor development (–0·05 [–0·15 to 0·06], p=0·36). There was moderate heterogeneity in expressive language outcomes (I^2=55·44%, p=0·028) and fine motor outcomes (I^2=57·58%, p=0·021), high heterogeneity in cognitive outcomes (I^2=70·95%, p=0·0011) and receptive language outcomes (I^2=67·91%, p=0·0047), and no heterogeneity in gross motor outcomes (I^2=0%, p=0·85). Sensitivity analyses

excluding the one study from a high-income country,[14] or using aggregate mean raw scores, where available, instead of standardised scores, resulted in similar estimates. Post hoc, on age stratification (age 12 and 24 months), expressive language differences were only apparent at 24 months, which reduced the heterogeneity (12 month effect size –0·16 [–0·42 to 0·11], p=0·24; heterogeneity I^2=72·23%, p=0·013, versus 24 month –0·19 [–0·27 to –0·11], p<0·0001; heterogeneity I^2=0%, p=0·51), whereas gross motor outcomes showed similar findings at both ages.

For the second aim, we included 13 articles (10 studies)[12–14,33,42–50] examining the effect of maternal ART regimens on child neurodevelopment (figure 4; appendix pp 23–24). Of these, four articles were from the Surveillance Monitoring for ART Toxicities (SMARTT) protocol of the Pediatric HIV/AIDS Cohort Study. Most studies used a cohort design; only two studies randomised ART regimens.[12,45] We were unable to do a meta-analysis given the regimen heterogeneity across studies.

Small studies comparing neurodevelopment of HEU children exposed to triple ART versus no ART in the first 24 months found similar outcomes[33,42] as did larger studies examining triple ART versus monotherapy from ages 1–5 years[13,43,12] (figure 4). Although one study in Botswana reported better neurodevelopment with triple ART compared with zidovudine only, this attenuated after adjustment for confounders.[43] Reports from the SMARTT cohort showed no evidence of differences in neurodevelopment at ages 12 or 24 months between children exposed to combination ART (defined as at least three drugs from at least two different drug classes) and non-combination ART regimens (including three nucleoside reverse transcriptase inhibitors [NRTIs], one or two drugs, or no ART);[14,44] protease inhibitor-containing or non-nucleoside reverse transcriptase inhibitor-containing (NNRTI) regimens compared with NRTI-only regimens;[14] protease inhibitor-containing versus no protease inhibitors and NNRTI-containing versus no NNRTIs;[44] and triple NRTI versus combination ART regimens.[50] Similarly, a randomised study from Botswana reported no neurodevelopmental differences comparing triple NRTI with dual NRTI plus protease inhibitor regimens at 24 months.[45]

Analyses of individual drugs were predominantly reported from the SMARTT cohort; of the multiple drugs assessed, most had no evidence of significant associations with neurodevelopment. However, there was a signal for worse language outcomes in children exposed to atazanavir-containing versus non-atazanavir regimens at age 12 months,[14] particularly when initiated in the second or third trimester,[47] although this was no longer apparent at age 24 months.[44] The Tshipidi-plus study from Botswana found HEU children who were exposed to efavirenz-containing regimens had lower performance-rated receptive language on the Bayley Scales of Infant and Toddler Development third edition at age 24 months compared with non-efavirenz-containing regimens. However, caregiver-rated language scores were higher in the efavirenz group.[49]

In the analysis of secondary outcomes (table 1; appendix pp 25–26), 11 reports from ten studies compared head circumference between HEU children and HU children.[13,28–30,39,51–56] Results were heterogeneous; five of ten studies found no difference between the two groups at ages 0–36 months.[13,28–30,51,53] One small study found a difference among neonates but not at later ages.[39] Beyond the neonatal period, the four largest studies (≥400 children each; ages 0–24 months), all from Africa, found that HEU children had significantly lower Z scores than HU children,[52,54–56] which held on adjusted analyses. Two reports found no relationships between combination ART regimens and head circumference (table 2);[57,58] however, in the SMARTT cohort, tenofovir[59] and atazanavir[47] were associated with smaller head circumference at age 1 year but not at age 2 years,[60] whereas efavirenz was associated with microcephaly, and poorer neurodevelopment in children with microcephaly in the first 5 years.[61] Estimates of microcephaly varied from 1% of HEU children[52] to 7·5%.[57] Only one neuroimaging study in South Africa compared HEU neonates and HU neonates: brain microstructural differences were identified, along with correlations between white matter microstructure and neurobehaviour.[62]

Discussion

We systematically reviewed neurodevelopment in HEU children aged 0–5 years and identified 45 reports from 31 studies across four continents; most studies were from sub-Saharan Africa, where the majority of HEU children live. Although findings were heterogeneous, over half of all studies reported poorer neurodevelopment in HEU children than in HU children in at least one domain. Variability in study design, quality, population characteristics, and assessment tools might explain differences in results across studies. Among high-quality studies, in which 99% of HEU children were exposed to maternal ART, there was evidence that HEU children have poorer expressive language and gross motor function than HU children, although the deficits are subtle with relatively small effect sizes. Timing of assessment appears to affect findings, with language deficits becoming evident after age 12 months, highlighting the importance of long-term follow-up. We found no evidence of consistent associations between specific ART regimens and neurodevelopment, although generalisability of the evidence is limited.

Our findings of impaired expressive language and gross motor development in HEU children are consistent with studies from before the introduction of ART.[5] Language problems have long been recognised in children with HIV,[63] with language expression more affected than comprehension.[24,64] Furthermore, a previous meta-analysis in HEU children including studies from before ART was

	Country (time period)	ART regimen*		Age at assessment	Tool	Unadjusted analysis outcomes†					Adjusted analysis
		Group 1	Group 2			CD	LD	MD	AB	SEB	
Triple ART *vs* no ART											
Familiar et al (2018)[33]	Uganda (2012–15)	ART (n=57)	No ART (n=18)‡	6 and 12 months	MSEL						Only adjusted results reported
Rajan et al (2017)[42]	India (2013–15)	ART (n=31)	No ART (n=10)	6–18 months	DASII						..
Triple ART *vs* zidovudine monotherapy											
Springer et al (2018)[13]	South Africa (2012–13)	ART (n=29)	Zidovudine (n=29)‡	12 months§	BSID-III & ADBB						..
Chaudhury et al (2018)[43]	Botswana (2006–08; 2010–12)	ART (n=382)	Zidovudine (n=210)	24 months	BSID-III						Significance lost on all domains
					DMC						
Boivin et al (2019)[12]	Malawi and Uganda (2013–14)	Group 1 (n=93) & group 2 (103) on antenatal ART+ postnatal triple ART or infant nevirapine	Group 3 (n=88) & group 4 (n=80)‡ on antenatal zidovudine + postnatal triple ART or infant nevirapine	12, 24, and 48 months	MSEL						Overall results held on longitudinal adjusted analyses with a few regimen differences¶
				48 and 60 months	KABC-II						
Comparison by drug regimen and class (PI, NRTI, or NNRTI)											
Rice et al (2013)[44]	USA and Puerto Rico (2007–11)	cART	Non-cART	12 months (n=535)	CDI (12 month)						Only adjusted analyses reported
		PI	No PI	24 months (n=503)	ASQ (24 month)						
		NNRTI	no NNRTI								
Sirois et al (2013)[14]	USA and Puerto Rico (2007–11)	cART	Non-cART	12 months (n=374)§	BSID-III						Only adjusted analyses reported
		PI-containing (+/- NNRTI)	NRTI only								
		NNRTI-containing (without PI)	NRTI only								
Kacanek et al (2018)[45]	Botswana (2006–08)	Triple NRTI (n=101)‖	Dual NRTI + PI (n=96)‖	24 months	BSID-III						Results held
					DMC & PSED						
Smith et al (2017)[46]	Canada (not reported)	Triple therapy, PI-based (n=43)	Triple therapy, NNRTI-based (n=16)	3·5 years**	WPPSI; VABS; VMI						..
Individual antiretroviral drugs: atazanavir focus											
Caniglia et al (2016)[47]	USA and Puerto Rico (2006–13)	ART: atazanavir-based 1st trimester initiation	ART: multiple, no atazanavir	9–15 months (n=575)	BSID-III						Only adjusted analyses reported
		Atazanavir-based 2nd and 3rd trimester initiation	ART: multiple, no atazanavir		BSID-III						
Rice et al (2013)[44]	USA and Puerto Rico (2007–11)	Individual drugs: atazanavir+	Individual drugs: no atazanavir	12 months (n=464)	CDI (12 months)						Only adjusted analyses reported††
				24 months (n=431)	ASQ (24 months)						

(Figure 4 continues on next page)

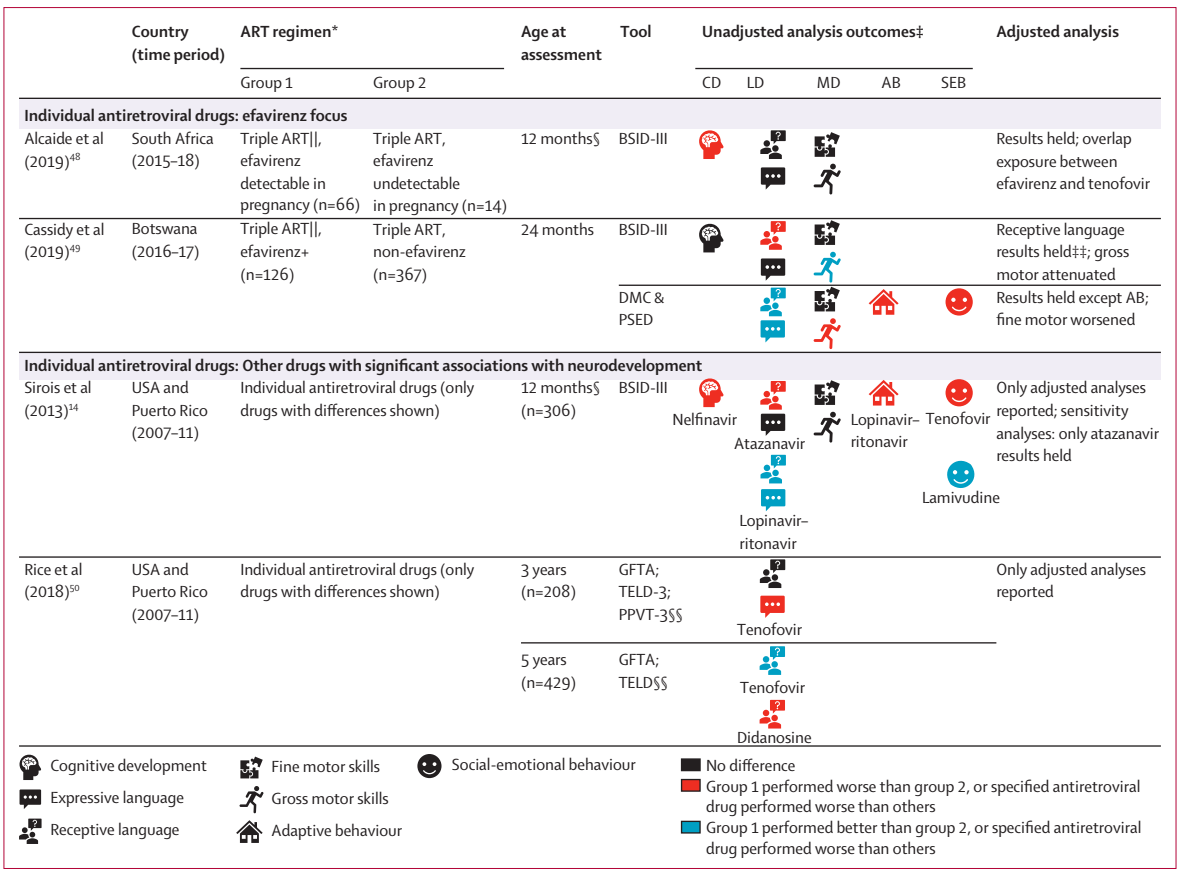

Figure 4: **Differences in neurodevelopment of HIV-exposed uninfected children by maternal ART**
CD=cognitive development. LD=language development. MD=motor development. AB=adaptive behaviour. SEB=social-emotional behaviour. HEU=HIV-exposed uninfected. HU=HIV-unexposed. ART=antiretroviral therapy. NNRTI=non-nucleoside reverse transcriptase inhibitor. NRTI=nucleoside reverse transcriptase inhibitor. PI=protease inhibitor. ASQ=Ages & Stages Questionnaire. BSID-III=Bayley Scales of Infant & Toddler Development 3rd edition. CDI=MacArthur-Bates Communicative Development Inventories. DASII=Development Assessment Scale for Indian Infants. DMC=Developmental Milestones Checklist. GFTA=Goldman-Fristoe Test of Articulation. KABC-II=Kaufman Assessment Battery for Children 2nd edition. MSEL=Mullen Scales of Early Learning. PPVT-3=Peabody Picture Vocabulary Test 3rd edition. PSED=Personal, Social and Emotional Development. TELD-3=Test of Early Language Development 3rd edition. VABS=Vineland Adaptive Behaviour Scales. VMI=Visual Motor Integration. WPPSI=Wechsler Preschool and Primary Scale of Intelligence. cART=combination ART defined in the SMARTT cohort as three or more drugs from two or more antiretroviral classes. *Number (n) given refers to the first visit in studies with multiple time-points, unless otherwise stated; group numbers differ across domains and ages and where multiple different drugs were assessed. †Unadjusted analysis outcomes defined by statistical significance of p<0·05 or through 95% CIs in group comparisons of the mean or comparison of delay where applicable. Where unadjusted analyses were not reported, adjusted analyses are presented instead. ‡These studies also had HU child groups; see figure 2. §Age is given as 12 months if median age of assessment fell within 1 month of this timepoint (appendix pp 23–24). ¶At age 4 years, MSEL cognitive composite scores were higher for children of mothers on antenatal and postnatal triple ART versus children of mothers not on triple ART consistently. ||Kacanek and colleagues[45] regimen: abacavir/zidovudine/lamivudine versus lopinavir–ritonavir/zidovudine/lamivudine; Alcaide and colleagues[48] and Cassidy and colleagues[49] efavirenz regimens: efavirenz/tenofovir/emtricitabine. **Stratified results for the age-point over 5 years are not presented due to review inclusion criteria. ††Multiple individual drugs assessed. At age 12 months, atazanavir increased odds of late language emergence (especially started in 2nd and 3rd trimester). Saquinavir had a similar effect although significance was lost on sensitivity analyses. Other drugs did not have significant associations. ‡‡Conception and 1st trimester efavirenz exposure worse that 2nd and 3rd trimester. §§Language impairment assigned as receptive language; speech impairment assigned as expressive language.

available found motor function was affected.[7] Therefore, despite reduced maternal morbidity and mortality due to ART, the negative effects of HIV exposure on language and motor skills remain. Expressive language and gross motor function are measured less often at older ages,[65] representing an important research gap. However, one US study identified language problems into adolescence, suggesting impairments might persist.[66] Given that early language predicts school performance,[67,68] and early motor skills influence other facets of development,[69] longitudinal follow-up is crucial.

There was no evidence of cognitive impairment in the meta-analysis, consistent with studies reporting similar cognitive scores between HEU children and HU children at age 6–11 years.[65] Although another meta-analysis reported that cognitive domains are affected in young HEU children,[7] this assessed the mental development index which combines cognitive and language development; our findings show the importance of separating individual neurodevelopmental domains. Even within our analyses there was high heterogeneity across assessments of the cognitive domain. Furthermore, the

	Country (time period)	HEU children*	HU children*	Assessment by age†	Adjusted analyses and comments
Head circumference of HEU children *vs* HU children					
Donald et al (2017)[51]	South Africa (2012–15)	131	536	No effect at birth	Results held on adjusted analysis
Filteau et al (2011)[55]	Zambia (2005–09)	125	382	HEU children had smaller head circumference at 6 months	Baseline trial results reported
Le Roux et al (2019)[52]	South Africa (2013–16)	461	411	HEU children had smaller head circumference at birth, 3 months, 9 months, and 12 months; no effect at 6 months	Results held on adjusted analysis; at 12 months 1% of HEU children had microcephaly and 17% had macrocephaly; of HU children 1% had microcephaly and 22% macrocephaly
Laughton et al (2012)[30]	South Africa (2005–06)	28	34	No effect at 12 months	..
Neri et al (2013)[53]	USA (2006–09)	82	82	No effect at average age 10 months (age range was 2 weeks to 2 years)	Results held on adjusted analysis
Springer et al (2018)[13]	South Africa (2012–13)	58	38	No effect at 12 months	
Jumare et al (2019)[54]	Nigeria (2013–17)	297	103	HEU children had smaller head circumference from birth to 18 months	Longitudinal analyses; lower head circumference-for-age Z score results held on adjusted analysis
Gomez et al (2009)[39]	Colombia (not reported)	23	20	HEU children had smaller head circumference at birth; no effect at 3, 6, 9, 12, and 24 months	..
Aizire et al (2020)[56]	Malawi and Uganda (2013–14)	471	462	No effect at 12 months; HEU children had smaller head circumference at 2 years in Uganda; no effect at 2 years in Malawi	Results held on adjusted analysis; risk of head circumference-for-age Z score less than WHO median increased among HEU children *vs* HU children at 24 months
Springer et al (2012)[28]	South Africa (2009)	17	20	No effect at 17–19 months	..
Springer et al (2020)[29]	South Africa (2012–13)	32	27	No effect at 30–42 months	4 (12·5%) HEU children had macrocephaly
Neuroimaging of HEU children *vs* HU children					
Tran et al (2016)[62]	South Africa (2012–15)	15	22	HEU children had altered neuroimaging findings at birth compared with HU children	Diffusion tensor imaging; altered white matter microstructure showing higher fractional anisotropy in the middle cerebellar peduncles of HEU children compared with HU children on adjusted analyses; higher fractional anisotropy in the left uncinate fasciculus correlated with abnormal neurological scores of HEU children

HEU=HIV-exposed uninfected. HU=HIV-unexposed. *Number (n) given refers to the first visit in studies with multiple timepoints. †Assessment by age defined by statistical significance of p<0·05 or through 95% CIs in group comparisons of the mean or comparison of delay using dichotomised variables.

Table 1: **Head circumference and neuroimaging outcomes of HEU children compared with HU children (aim 1)**

restricted analysis of high-quality studies in their meta-analysis[7] showed no cognitive differences between HEU children and HU children, portraying the importance of focusing on studies with low bias. Social-emotional and adaptive behaviour, defined as living skills that enable everyday function, did not differ between the two groups in high-quality studies in our review. However, the low number of studies reporting on these outcomes prevent reliable conclusions being drawn; further investigation is needed.

WHO guidelines changed in 2012 to recommend universal triple ART for pregnant and breastfeeding women (termed Option B+). To our knowledge, this is the first systematic review to assess the effect of specific ART exposure on the neurodevelopment of HEU children. Overall, our results are reassuring, showing no clear evidence of associations between different ART regimens and drug classes assessed (ie, triple therapy, monotherapy, NRTI, NNRTI, or protease inhibitor-based) and neurodevelopment. However, interpretation of these results is limited by small study numbers and heterogeneous comparison groups. Data on individual drugs are dominated by publications from the observational SMARTT study, which includes children exposed to multiple different combinations. Overall, findings from SMARTT are encouraging,[70] albeit with some concerns regarding efavirenz[61] and atazanavir.[47] However, given the non-randomised design in a US

	Country (time period)	Group A*	Group B*	Assessment by age†	Adjusted analyses and comments
Spaulding et al (2016)[57]	Latin America and Caribbean (2002–09)	Multiple ART combinations (n=1400)	..	No effect on head circumference at 6–12 weeks and 6 months	Microcephaly and neurological conditions assessed; no difference on timing of initiation of combination ART or specific drugs; microcephaly reported in 7·5% of HEU children
Pintye et al (2015)[58]	Kenya (2013)	Triple ART with tenofovir (n=51)	Triple ART without tenofovir (n=104)	No effect on head circumference at 6 weeks and 9 months	No associations between prenatal tenofovir use and head circumference-for-age Z score in 6-week or 9-month infant cohorts.
Siberry et al (2012)[59]	USA and Puerto Rico (2005–10)	Triple ART with tenofovir (n=274)	Triple ART without tenofovir (n=416)	No effect on head circumference at birth; tenofovir associated with smaller head circumference at 12 months	Results held on adjusted analysis
Caniglia et al (2016)[47]	USA and Puerto Rico (2006–13)	ART with atazanavir (n=127)	ART without atazanavir (n=525)	Atazanavir associated with smaller head circumference at 12 months	Results held on adjusted analysis; overlap between atazanavir and tenofovir in regimens
Jacobson et al (2017)[60]	USA and Puerto Rico (2007–11)	Triple ART, multiple drugs (n=509)	..	No effect on head circumference at 2 years	No difference by ART regimen or timing of initiation on unadjusted or adjusted analyses; compared tenofovir, atazanavir, nelfinavir, and boosted protease inhibitor regimens
Williams et al (2020)[61]	USA and Puerto Rico (2007–17)	Individual drugs (n=3055); ART with efavirenz (n=141)	ART without efavirenz (n=2842)	Efavirenz associated with smaller head circumference; microcephaly assessed	Efavirenz exposure was associated with increased risk of microcephaly on adjusted analysis; no difference preconception or postconception initiation; more pronounced associations with efavirenz regimens containing zidovudine plus lamivudine compared to tenofovir plus emtricitabine; protective associations with darunavir; increased risk with fosamprenavir; microcephaly was associated with worse neurodevelopment in all domains; multiple drugs assessed and efavirenz was the association reported that survived in the fully adjusted model

ART=antiretroviral therapy. HEU=HIV-exposed uninfected. *Number (n) given refers to the first visit in studies with multiple timepoints. †Assessment by age defined by statistical significance of p<0·05 or through 95% CIs in group comparisons of the mean or comparison of delay using dichotomised variables.

Table 2: **Head circumference by different maternal ART (aim 2)**

population, it is unclear how generalisable these findings are. An observational study from Botswana reported conflicting results for efavirenz exposure and language,[49] suggesting further evaluation is needed, with ongoing pharmacovigilance to monitor new drugs.

Secondary outcomes included head circumference, which is often used as a surrogate marker of brain growth and development and might serve as a useful biomarker in clinical practice. Our findings were mixed, and the prevalence of microcephaly varied across studies, suggesting that more robust evidence is needed with long-term follow-up. However, larger studies did suggest a small reduction in head circumference in HEU children compared with HU children. There was no evident association between head circumference and specific ART regimens or drug classes; however, potential associations with efavirenz[61] were reported from the SMARTT cohort. The SMARTT study also found a relationship between microcephaly in HEU children and neurodevelopmental impairment, which needs to be explored further. Only one neonatal study included neuroimaging and identified white matter alterations associated with adverse neurodevelopment in HEU children compared with HU children, suggesting a potential neurological pathway,[62] which paves the way for further work. We did not specifically evaluate mechanisms in our review, but others have discussed this in detail.[71]

Our study had several strengths. We reviewed multiple neurodevelopmental outcomes among HEU children up to age 5 years, from diverse contexts across several continents. The meta-analysis addressed sample size and methodological weaknesses of individual studies. We used effect sizes to combine measurement tools, allowing us to identify vulnerability in specific domains and to estimate a measure of effect that could be applied to different tools and ages in the future. Health-care workers can be guided by the effect sizes and examine for small delays in development in this group of children. Our study builds on previous reviews[5–7] with larger, higher-quality African studies included in the meta-analysis; furthermore, we specifically focused on ART and neuroimaging. Several limitations and research gaps were identified (panel). There was substantial heterogeneity across study designs,

***Panel*: Methodological considerations for future studies examining the neurodevelopment of HEU children**

Given the known effect of multiple factors on neurodevelopment, a more coherent approach is needed in which a unified set of covariates are measured and reported with adequate comparator groups to ensure consistency across studies and allow for generalisability.

A standardised framework for assessments with validated cross-cultural measurements is required to improve comparability between different study settings with contextually appropriate norms. Reporting categorical delay scores is useful from a clinical perspective; however, the selection of the threshold is often arbitrary and might miss capturing the full relationship. Therefore, reporting both continuous and categorical measures in tandem might be the best approach.

Individual neurodevelopmental domains should be examined separately. The use of multiple tools for assessing the different components of domains should be considered. This is particularly relevant given the language deficits identified in HEU children, since multiple indicators for speech and language might differentiate between vocabulary, grammar, and speech. As children grow older, differentiating between the various components of executive function also becomes increasingly important.

The definitions of HEU and HIV-unexposed need to be carefully documented. We had to exclude many studies that combined HEU children and children with HIV infection together as HIV-exposed, or which combined children with and without HIV exposure together as HIV-uninfected. In the first instance, differences are likely to be overestimated due to the inclusion of children with HIV, and in the latter, differences might be underestimated due to the inclusion of children without HIV exposure.

Studies should consider factors that are known to affect child neurodevelopment at the design stage (population criteria or covariates), such as low birthweight and preterm birth, small-for-gestational age, hearing impairment, genetic syndromes, and neurological disorders.

Ongoing ART surveillance is needed and randomised controlled trials might help to identify the ART regimens that lead to optimal outcomes. This requires examination of duration of exposure (including ART exposure antenatally and postnatally through breastfeeding, and child prophylaxis) with adequate comparison groups of specific regimens.

Given the dynamic nature of neurodevelopment, the developmental trajectory of HEU children compared with their unexposed peers should form the basis for future work. More large-scale longitudinal studies are needed given the potential subtle deficits early in life, and follow-up is required to determine longer-term effects.

ART=antiretroviral therapy. HEU=HIV-exposed uninfected.

sample sizes, measurement tools, blinding, quality, and population demographics; only half of the studies included adjusted analyses, and confounder selection varied. Other contributors to child development were not assessed, including vision and hearing loss, which has been reported in older HEU children.[72] Given the heterogeneity in studies, the results of the meta-analysis should be treated with caution and a causal relationship should not be assumed due to the potential for confounding. However, combining studies with low risk of bias is acceptable, even in the presence of statistical heterogeneity.[15] We used random-effects models, and combined results across different tools, which is appropriate in the early years (age 0–3 years) in which neurodevelopment has more global commonalities in skill development than in later years.[73,74] Evidence is scarce for the analysis of ART. Results for individual antiretroviral drugs were predominantly from one US observational study, which limits generalisability. Due to a paucity of study reporting, we were unable to assess the effect of timing of maternal ART initiation that might have influenced infant outcomes. Only two studies randomised ART use,[12,45] and newer drugs including integrase inhibitors were not assessed.[75] The reliance on observational data provides several challenges in interpretation. Studies had difficulty separating specific drugs, and ART comparison groups often comprise multiple different antiretroviral combinations. Furthermore, maternal ART initiation might be a proxy for HIV disease severity, and mothers might change regimens during pregnancy.

In conclusion, early-life neurodevelopment of HEU children is modestly impaired, specifically in expressive language and gross motor domains. Although effect sizes were small, our findings suggest a subtle yet clear demarcation of differences in abilities between HEU children and HU children at young ages. Given the growth in the global population of HEU children, with the largest increase in Africa, this difference in neurodevelopmental function might have a substantial effect together with other risks faced by this population. There were no consistent signals that specific ART regimens or drug classes affect neurodevelopment; however, evidence is scarce and well designed randomised trials are required. Understanding the potential toxicities or relative neuroprotection of antiretrovirals would allow modification of maternal ART regimens to optimise infant neurodevelopment. There was a scarcity of neuroimaging studies, and our review raises some important methodological considerations for future studies examining neurodevelopment in HEU children (panel). Greater understanding of neurodevelopment in this population will aid identification of vulnerable infants to allow early prevention and intervention strategies during developmentally sensitive periods. The apparent detriment in

early motor and language skills among these children calls for more longitudinal studies to investigate neurodevelopmental trajectories, delineate mechanisms, and inform recommendations to support this growing population.

Contributors

CJW was responsible for the conceptualisation, methodology, formal analysis, investigation, data curation, and writing (original draft preparation, and review and editing). EW was responsible for the methodology, investigation, validation, data curation, and writing (review and editing). CB-C was responsible for the methodology, investigation, validation, visualisation, and writing (review and editing). AMR was responsible for the methodology, formal analysis, visualisation, and writing, (review and editing). DJS was responsible for the conceptualisation and writing (review and editing). DMG and SY were responsible for the conceptualisation, supervision, and writing (review and editing). AJP and KAD were responsible for the conceptualisation, methodology, investigation, supervision, and writing (review and editing). CJW, CB-C, and AMR accessed and verified the data. All authors had access to the data and accept responsibility to submit for publication.

Declaration of interests

AJP declares paid participation on the Botnar Research Centre for Child Health independent external review board and is a member of several data and safety monitoring boards with no payment, none of which relate to the current research. DJS has received research grants or consultancy honoraria from Discovery, Johnson & Johnson, Lundbeck, Sanofi, Servier, Takeda, and Vistagen. All other authors declare no competing interests.

Data sharing

The study protocol and materials are available at https://osf.io/yeqj3/ and in the appendix (pp 2–26).

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Part III

Neuroimaging

7

Chapter 7: Neuroimaging Young Children and Associations with Neurocognitive Development in a South African Birth Cohort Study

(Research paper)

Neuroimaging young children and associations with neurocognitive development in a South African birth cohort study

Summary

Chapter 7 presents the fourth research paper titled 'Neuroimaging young children and associations with neurocognitive development in a South African birth cohort study'. This paper introduces the neuroimaging part of the thesis, building on the findings of poorer neurodevelopment in HEU children in the prior section. The manuscript describes the neuroimaging methods of the study and the rationale for neuroimaging as an indispensable tool for investigating brain development in young children and the neurobiological mechanisms underlying developmental risk and resilience. However, the challenges of paediatric MRI imaging, particularly motion sensitivity, have limited neuroimaging in this age group, resulting in scarce MRI research from SSA to date.

This paper outlines the methods we used to overcome these challenges and successfully undertake neuroimaging at age 2-3 years in the DCHS, demonstrating the feasibility of neuroimaging young children during natural sleep in SSA. While the neonatal imaging is not described, a similar method was used to scan infants in the first weeks of life without sedation. The research sets the scene for the following chapters (8, 9, and 10) that examine the effect of HIV exposure on child brain structure, addressing objective 2. Finally, the paper investigated the associations between regional cortical structure (surface area and thickness) and cognitive and language development at this young age. The findings indicate that dynamic morphological changes in heteromodal association regions are associated with neurocognitive development, providing relevant background information to explore the impact of HIV exposure on the brain structure-function relationship in objective 3.

My role encompassed all aspects of the neuroimaging processes, including developing, co-ordinating and conducting the imaging of the 2-3 year olds. This involved working with the CUBIC radiographers to optimise the imaging and with a sleep management specialist to improve the scan success rate; organising the logistics of the scans by arranging recruitment and transport from Paarl to Groote Schuur Hospital for the participants (60km each way); training the research nurses; and setting up a formal clinical reporting system. I was responsible for the data collection with supervision from Prof Kirsty Donald and the scanning sessions were a wonderful team effort including Sivenesi Subramoney (research assistant), the research nurses, the DCHS team, and the radiographers. After completing FreeSurfer imaging training, I undertook the data processing with Sivenesi Subramoney and advice from Dr Jean-

Paul Fouche (imaging expert). I conceived and performed the statistical analysis with advice from A/Prof Andrea Rehman (statistician) and Dr Shantanu Joshi and Prof Katherine Narr (UCLA imaging). I wrote the first draft of the paper and incorporated feedback from co-authors and peer reviewers.

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Supplementary material

The supplementary material as detailed in the published article is available at https://doi.org/10.1016/j.neuroimage.2020.116846 and listed in Appendix VII.

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Neuroimaging young children and associations with neurocognitive development in a South African birth cohort study

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ABSTRACT

Magnetic resonance imaging (MRI) is an indispensable tool for investigating brain development in young children and the neurobiological mechanisms underlying developmental risk and resilience. Sub-Saharan Africa has the highest proportion of children at risk of developmental delay worldwide, yet in this region there is very limited neuroimaging research focusing on the neurobiology of such impairment. Furthermore, paediatric MRI imaging is challenging in any setting due to motion sensitivity. Although sedation and anesthesia are routinely used in clinical practice to minimise movement in young children, this may not be ethical in the context of research. Our study aimed to investigate the feasibility of paediatric multimodal MRI at age 2–3 years without sedation, and to explore the relationship between cortical structure and neurocognitive development at this understudied age in a sub-Saharan African setting. A total of 239 children from the Drakenstein Child Health Study, a large observational South African birth cohort, were recruited for neuroimaging at 2–3 years of age. Scans were conducted during natural sleep utilising locally developed techniques. T1-MEMPRAGE and T2-weighted structural imaging, resting state functional MRI, diffusion tensor imaging and magnetic resonance spectroscopy sequences were included. Child neurodevelopment was assessed using the Bayley-III Scales of Infant and Toddler Development. Following 23 pilot scans, 216 children underwent scanning and T1-weighted images were obtained from 167/216 (77%) of children (median age 34.8 months). Furthermore, we found cortical surface area and thickness within frontal regions were associated with cognitive development, and in temporal and frontal regions with language development (beta coefficient $\geq$0.20). Overall, we demonstrate the feasibility of carrying out a neuroimaging study of young children during natural sleep in sub-Saharan Africa. Our findings indicate that dynamic morphological changes in heteromodal association regions are associated with cognitive and language development at this young age. These proof-of-concept analyses suggest similar links between the brain and cognition as prior literature from high income countries, enhancing understanding of the interplay between cortical structure and function during brain maturation.

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1. Introduction

The first three years of life represent the most extensive period of brain growth and synapse development, where critical neural pathway development and network maturation occur (Hermoye et al., 2006; Ouyang et al., 2019; Knickmeyer et al., 2008). This early brain development shapes each child's future potential and is critical to later educational outcomes and human capital (Walker et al., 2011; Daelmans et al., 2017). The United Nations Sustainable Development Goals (SDGs, https://sustainabledevelopment.un.org) have focused the world on the importance of children thriving; however, 43% of children under 5 years are at risk of failing to reach their developmental potential worldwide. The majority of these children live in low and middle-income countries (LMIC); sub-Saharan Africa (SSA) has the highest proportion of children at risk of developmental delay (Black et al., 2017).

Magnetic resonance imaging (MRI) has revolutionised our ability to examine brain structure, function and connectivity, and emergent technologies have allowed increasingly sophisticated investigation into neuroanatomy and neurocircuitry. MRI has many advantages over other imaging modalities including high image resolution without radiation exposure, offering a safe tool for investigating early brain development in young children and the neurobiological mechanisms behind developmental delay. However, MRI studies of young children in LMICs are lacking (Azhari et al., 2019) and very little research has been performed to assess the link between early brain structure and cognitive development in SSA (Paterson et al., 2006).

Studies from high income countries have used MRI to examine brain development in neonates, school-aged children, and adolescents (Wierenga et al., 2018; Hagler et al., 2019). However, only a few have investigated brain structure and function in preschool children between the ages of 1–3 years when cortical maturation is most rapid, despite recognition of the importance of this period in terms of structural and functional development (Gilmore et al., 2018). Beyond early infancy, paediatric imaging is difficult due to the MRI requirement of lying still in an enclosed space for a prolonged period of time (Barkovich et al., 2019) leading to technical and practical challenges (Almli et al., 2007; Raschle et al., 2012; Thieba et al., 2018). This is particularly notable in preschool children who are not able to understand the reasons for this requirement (Raschle et al., 2012; Thieba et al., 2018) and are not responsive to mock scanner training which can be effective for older children (Thieba et al., 2018). Although sedation and anesthesia are frequently used in clinical practice, there are risks associated with these approaches that have ethical implications for their use in research (Edwards and Arthurs, 2011; Jevtovic-Todorovic et al., 2013; Cote et al., 2000; Sammons et al., 2011). These techniques may also dynamically affect brain signal during functional imaging (Jevtovic-Todorovic et al., 2013).

Previous studies from high income countries in infancy and later childhood have found that distinct brain areas are associated with various neurodevelopmental functions, including areas of the frontal lobe associated with cognitive development (Paterson et al., 2006; Lyall et al., 2015; Shaw et al., 2006) and frontal and temporal regions associated with language (Imada et al., 2006; Dehaene-Lambertz et al., 2010). There is a need for studies to investigate the brain structure-cognition relationship across different socio-cultural contexts (Azhari et al., 2019) and in LMICs where the majority of children at-risk of developmental impairment reside (Tomlinson et al., 2014). Neuroimaging from a young age may provide insight into early neurodevelopment processes as well as the relationships with current and future neuropathology (Gilmore et al., 2018; Jahanshad et al., 2015; Gao et al., 2019).

The Drakenstein Child Health Study is a large population-based birth cohort study in the Western Cape of South Africa investigating the early life determinants of child health (Stein et al., 2015; Zar et al., 2015). This cohort study offered a unique opportunity to investigate associations between early brain structure and neurodevelopment. In this prospective study, we aimed firstly to establish the feasibility of performing paediatric multimodal MR scanning during natural sleep at age 2–3 years in a sub-Saharan African setting; secondly to assess the association between scan success and neurodevelopment; and thirdly to explore the relationships between regional cortical structure and cognitive and language development at this young age, important predictors of later cognitive outcome.

2. Materials and methods

2.1. Study design

The Drakenstein Child Health Study (DCHS) is an observational population-based birth cohort in South Africa (Stein et al., 2015; Zar et al., 2015). Women were recruited during pregnancy and mother-child pairs are followed up longitudinally. Neuroimaging was performed as a prospective nested study.

2.2. Study setting

The DCHS study is located in the Drakenstein sub-district, a peri-urban area 60 km outside Cape Town, Western Cape, South Africa. This sub-district has a high burden of infectious diseases and psychosocial stressors representative of many other LMICs (Stein et al., 2015). Pregnant women were recruited into the DCHS from two public sector primary health care clinics: Mbekweni (serving a predominantly black African community) and TC Newman (serving a mixed ancestry community) between March 2012 and March 2015.

2.3. Participants

2.3.1. Drakenstein Child Health Study

Mothers were enrolled into the DCHS at 20–28 weeks' gestation while attending routine antenatal care using an unfiltered approach to ensure the cohort was representative of the local population. Pregnant women were eligible for the study if they were 18 years or older, attended one of the recruitment clinics and intended to remain in the area. Mothers provided written informed consent at enrolment and are re-consented annually. Consent was done in the mother's preferred language: English, Afrikaans or isiXhosa. Between May 2012 and September 2015, there were 1143 live infants in the umbrella DCHS. In the catchment area of the Drakenstein sub-district where the majority of births occur at Paarl Hospital, the study enrolled approximately 10% of births (Donald et al., 2018). This represents approximately 0.03% of all births in South Africa during this time period (Stats SA, 2016).

2.3.2. Neuroimaging sub-study

A sub-group of mother-child pairs were selected from the DCHS cohort for neuroimaging at 2–3 years of age with a pilot phase from July to December 2015, and the main study from January 2016 to September 2018. A total of 239 mother-child pairs were invited to attend for neuroimaging when the child turned 2 years who were known to be currently active in the cohort, staying in the study area, and had none of the following exclusion criteria: (i) Medical comorbidity (genetic syndrome, neurological disorder, or congenital abnormality); (ii) Gestation <36 weeks; (iii) Low Apgar score (<7 at 5 min); (iv) Neonatal intensive care admission; (v) Maternal use of illicit drugs during pregnancy; (vi) Child HIV infection. Children who underwent MRI in the neonatal period were prioritised; methods are described in full elsewhere (Donald et al., 2018). Children were selected for neuroimaging based on risk factor exposure to ensure adequate representation, and a randomly selected comparison group frequency matched by age and sex. Written informed consent was obtained from the parent.

2.4. Demographics

Sociodemographic data were collected in interviews at 28–32 weeks' gestation by trained study staff using validated questionnaires (Myer et al.,

2008). Measures of socioeconomic status (SES) included household income, maternal education and employment. In order to adequately capture variability of SES within this setting, we also standardised these measures along with an asset index and created an aggregate measure of SES (Myer et al., 2008), divided into quartiles. Study staff attended births and birth data were prospectively collected or abstracted from hospital records. Child gestational age was calculated from antenatal ultrasound where available, or symphysis-fundal height or maternal report of last menstrual period.

2.5. Clinical developmental outcomes

Child development was assessed using the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III) at 2 years of age (Bayley, 2006; Ballot et al., 2012). The BSID-III assessment is one of the most comprehensive tools to assess early child development. It is sensitive to subtle developmental delay across cognitive, language and motor scales (Bayley, 2006; Albers and Grieve, 2007), and has been validated in South Africa (Ballot et al., 2012; Rademeyer and Jacklin, 2013). The BSID-III was administered by trained assessors who used direct observation to assess child development and gave language prompts in the child's preferred language (Afrikaans or isiXhosa). Assessments were standardised to ensure concordance. Scoring was done according to the manual and BSID specialized software which produces norm-referenced scores across subscales. Standardised composite scores were calculated (mean 100, standard deviation [SD] 15) using normative values from a US reference population as per the assessment guidance to allow comparison across domains. Cognitive and language composite scores were included for this analysis as measures of neurocognitive function.

2.6. Neuroimaging

2.6.1. Data acquisition

Neuroimaging was performed at the Cape Universities Brain Imaging Centre (CUBIC). The pilot phase took place at Tygerberg Hospital from July to December 2015 on a 3T Siemens Allegra MRI scanner (Erlangen, Germany), where our group had experience with scanning neonates (Donald et al., 2015). From January 2016 to September 2018 the main neuroimaging sub-group (excluding the pilot group) had scanning performed at Groote Schuur Hospital on a research-dedicated 3T Siemens Skyra 70 cm diameter bore whole body MRI scanner (Siemens, Erlangen, Germany) (http://www.cubic.uct.ac.za). A 32-channel head coil optimised for young children was used with stabilising cushions to reduce head movement during scans. The full scan protocol included 1) MEMPRAGE T1 and T2-weighted structural imaging; 2) resting state functional imaging; 3) single voxel magnetic resonance spectroscopy; and 4) diffusion tensor imaging. The total scan duration was approximately 1 h (58 min 38 s) with slight variation depending on shimming. See Table 1 for sequence parameters and measures. Processing and analysis procedures described below are focused on the structural scans only.

The scans were undertaken during natural sleep utilising locally developed techniques: imaging was conducted after lunch or in the evening to coincide with routine sleep times. Mothers were asked to keep the child awake prior to the scan. On arrival, a full explanation of the MRI process was given, informed consent was taken from the parent/guardian and an MRI safety screening questionnaire was administered. Children were directed to a separate playroom equipped with toys and picture books with a member of the study team to maximise comfort and allow acclimatisation to an unfamiliar environment. The team adopted a child-friendly approach throughout the whole process to put the children at ease and incorporated play to reduce anxiety (Raschle et al., 2012). Children were encouraged to play quietly to encourage sleeping afterwards, and anthropometric data collection was integrated into this routine. The child and caregiver were given a warm meal, and the child was given melatonin (dosage 3–6 mg) (Ibekwe et al., 2017; Paediatric Formulary Committee, 2016) with yoghurt to improve the taste to promote sleep-initiation (Johnson et al., 2002). The children were wrapped warmly and encouraged to sleep in their natural position – either swaddled on their mother's back (common practice in this community) or lying on a bed in a low-lit room. Once the child had fallen into deep sleep, he/she was carried into the scanner, positioned carefully and ear protection was fitted. The scanner was padded with pillows and blankets and a trained study staff member remained in the scanner room throughout the scan to alert the radiographer if the child woke.

During acquisition, images were checked in real-time and if a substantial artefact was observed (most commonly due to movement), and there was sufficient time, then that specific sequence was repeated. Similarly, if the child woke during the scan protocol then he/she was encouraged to sleep again so the protocol could be completed. If further

Table 1
Imaging modalities and acquired parameters.

Sequence	Measures	Parameters: Siemens Skyra sequences
3D T1-weighted MEMPRAGE (Multi-Echo Magnetization Prepared Rapid Acquisition Gradient Echo)	Subcortical and cortical tissue volumes; Surface-wise measures including cortical thickness, surface area and gyrification.	Sagittal orientation; Repetition time (TR) = 2530 ms; echo time (TE) = 1.69, 3.54, 5.39, 7.24 ms; flip angle = 7.0°; voxel size 1.0 × 1.0 × 1.0 mm^3; inversion time (TI) = 1100 ms; field of view (FOV) = 224 × 224 × 176 mm; 176 slices, 1.0 mm thick. Scan time: 5min21s.
Resting state blood-oxygen-level dependent (BOLD) echo planar imaging (EPI)	Resting brain networks.	TR 2000 ms; TE 30 ms; flip angle = 77°, 33 slices, slice thickness 4 mm; slice gap 1 mm, voxel size 3.4 × 3.4 × 4.0 mm. FOV = 220 × 220mm, Scan time 8min04s.
Single voxel PRESS (Point RESolved Spectroscopy) magnetic resonance spectroscopy (MRS)	Relative metabolite concentrations of phosphocreatine (Cr + PCr), glutamate with glutamine (Glx), glutamate (Glu), n-acetyl-aspartate with n-acetyl-aspartyl-glutamate (NAA + NAAG), n-acetyl-aspartate (NAA), choline containing metabolites (glycerophosphocholine + phosphocholine [GPc + PCh]), and myo-inositol (mI).	*Voxel 1:* Midline parietal grey matter. *Voxels 2 and 3*: Left and right parietal white matter. TR = 2000 ms, TE = 30 ms (128 averages) with water references (TE = 30, 75, 100, 144, 500, 1000 ms); Voxel size 25 × 25 × 25 mm^3. Scan time: ~6 min total per voxel with water references.
Diffusion Tensor Imaging (DTI)	Fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD).	A pair of diffusion-weight datasets with opposite phase encoding (anterior-posterior and posterior-anterior) were acquired using 30 noncollinear gradient directions with DWfactor b = 1000 s/mm^2, and one non-DW b = 0s mm^2 (b_0) acquisition); TR = 7800 ms; TE = 92 ms; voxel size = 1.8 × 1.8 × 2.0 mm^3, FOV = 230 x 230 x 121 mm, slice thickness 2.0 mm, Scan time: 2 × 8min36s.
3D Sagittal T2-weighted structural imaging	Subcortical and cortical tissue volumes.	TR = 3200 ms; TE = 409 ms; FOV = 230 × 230 mm; voxel size = 0.9 × 0.9 × 1.0 mm^3; 160 slices, 1.0 mm thick. Scan time: 3min7s

sleep was not possible, with parental consent the child was brought back on a different day to attempt the scan again, up to a maximum of three times.

2.6.2. Scan reporting

Structural sequences were reviewed by a radiologist to check for clinical incidental findings and a formal report was provided to the study team. Any incidental findings were discussed with a paediatric neurologist. Relevant findings were discussed with the child's parents, and referred for management through established local clinical pathways as appropriate.

2.6.3. Processing and quality control overview

Imaging data processing included 1) quality checking to prepare the data and correct for motion artefact; 2) processing as per each individual modality to extract the relevant outcome measures; and 3) statistical analysis. Below we outline the processing conducted on the T1-weighted scans.

2.6.3.1. T1-weighted MR images processing and quality control. After the image acquisition, all T1-weighted MR images were processed using FreeSurfer version 6.0 software (http://surfer.nmr.mgh.harvard.edu/) utilising the automated techniques for cortical reconstruction and volumetric segmentation (Fischl et al., 2002, 2004; Dale et al., 1999; Desikan et al., 2006). Structural T1 images were first converted from DICOM to NIfTI format. Scans were then processed through the FreeSurfer programme using the recon-all command at the local supercomputing cluster at the Centre for High Performance Computing (CHPC, Cape Town) (https://www.chpc.ac.za). The pipeline involved skull stripping, B1 bias field correction, normalisation, grey-white matter segmentation, surface atlas registration and extraction, and automated cortical reconstruction producing regional and total brain volumes, and anatomical measures including cortical surface area and cortical thickness (Fischl and Dale, 2000). Cortical regions-of-interest (ROIs) (surface area and thickness) were extracted for analysis.

The T1-MEMPRAGE images were checked for movement and completeness. Images were visually quality checked for movement artefact. FreeSurfer outputs were also visually inspected for errors in segmentation of cortical and subcortical structures. Overall, FreeSurfer processing was not possible in one case, and one child had an artefact that failed correction after processing. There were no errors of segmentation of cortical structures, however, two children were found to have incidental findings on clinical report Using the ENIGMA pipeline (http://enigma.ini.usc.edu/protocols/imaging-protocols/), subjects were reviewed if ROIs in the final output were classified as extreme outliers (Nwosu et al., 2018; ENIGMA, 2019).

2.7. Ethics

The DCHS was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009) and by the Western Cape Provincial Health Research Committee. The imaging was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (525/2012) and the London School of Hygiene & Tropical Medicine (11903).

2.8. Statistical analyses

2.8.1. Scan success

Children were categorised as having a full scan (completed acquisition of all sequences), part successful scan (1–4 sequences), or no scan to quantify scan success. Developmental outcomes, as measured by the BSID-III, were calculated as standardised composite scores (continuous measures) and categorised into delay variables (using a standard cut-off of $<$ -1 SD from the BSID-III reference mean) reported as means and standard deviations and proportions respectively. Child clinical neurodevelopment, age and sex were associated with scan success using ANOVA.

2.8.2. Cortical structure and neurocognitive development

Analyses were conducted on n = 146 children who had complete data available for all relevant independent variables (age, sex, ROI) and at least one dependent clinical development measure (cognitive or language development). Sociodemographic and developmental outcomes were expressed as mean (SD) for continuous data and frequencies (%) for categorical data. Comparisons were made with the full DCHS cohort to assess generalisability using descriptive statistics (χ^2 test).

Cortical surface area and cortical thickness measurements were standardised and associated with composite developmental outcomes (cognitive and language development) from the BSID-III at 2 years. Multiple linear regression was used to model regional associations with neurodevelopmental outcomes, where cognitive development was the dependent variable, and cortical surface area or thickness were independent variables. Given the substantial variability of child developmental trajectories; the rapid development during this time meaning cognitive mapping may extend over multiple areas; and that few imaging studies have been performed at this age, we conducted an exploratory analysis examining all ROIs (Lyall et al., 2015). However, on the basis of prior literature, we hypothesised that areas of the frontal lobe would be associated with cognitive development, areas linked to language function would include frontal and temporal regions, and that there would be regions of shared cognitive and language function (Paterson et al., 2006). Age and sex were included as covariates in all analyses. Intracranial volume (ICV) was also added into the surface area analyses (Voevodskaya et al., 2014). We report standardised beta coefficients with 95% confidence intervals to illustrate effect sizes (Murner-Lavanchy et al., 2018; Nieminen et al., 2013). We focus on regions with an association with cognitive or language development with a beta coefficient $\geq$0.20 (a small-moderate effect size (Acock, 2014; Sawilowsky, 2009) and for illustrative purposes have constructed effect size maps with any regions with uncorrected $p < 0.05$.

Additional analyses were performed including household income as a confounder given reports that socioeconomic status may affect the brain-cognition relationship (Brito et al., 2017). A sensitivity analysis was also performed excluding children who were classified as outliers (ENIGMA, 2019). All analyses were performed with STATA version 14.0.

2.9. Data and code availability statement

The de-identified data that support the findings of this study are available from the authors upon reasonable request as per cohort guidelines.

3. Results

A total of 239 children attended for imaging at 2–3 years; 23/239 (9.6%) for the pilot at Tygerberg CUBIC, and 216/239 (90.4%) for the main neuroimaging study to Groote Schuur CUBIC (Fig. 1).

Of those children attending the main neuroimaging study at Groote Schuur CUBIC (n = 216), the median age was 34.8 months (IQR 33.7–35.6) and 122 (56%) were male. Children in the neuroimaging subgroup were representative of the full cohort at birth (n = 1143), and those in follow up at 2 years (n = 1002), with similar maternal educational attainment and overall socioeconomic status. However, a greater proportion of children in the neuroimaging sub-group were in the middle income category (Supplementary Tables 1 and 2).

3.1. Scan success

Overall, 167/216 children (77%) successfully completed at least T1-weighted scanning (Fig. 1). 49/216 (23%) children did not sleep and we

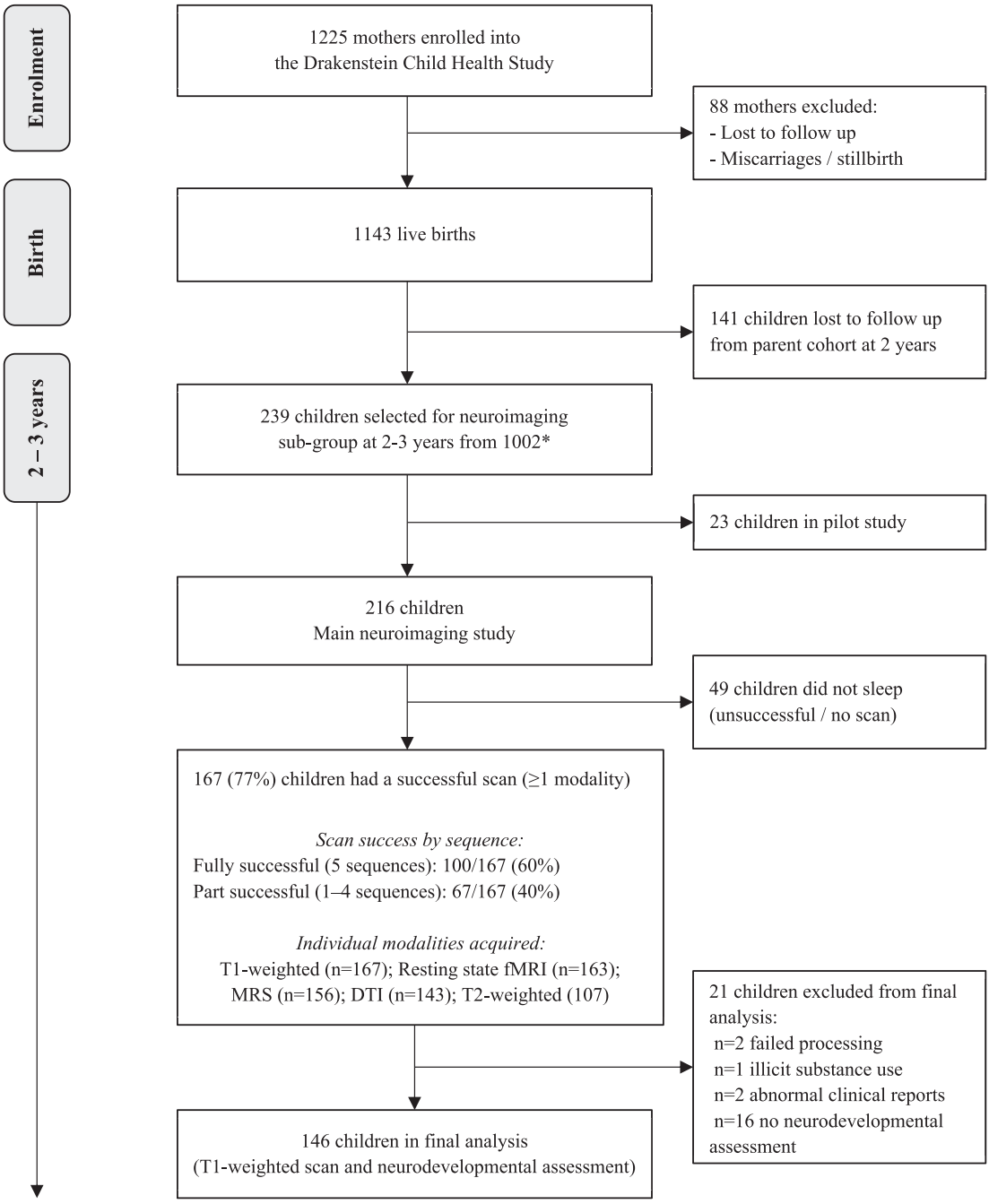

Fig. 1. Flow chart for neuroimaging in the DCHS cohort and sequence success at 2–3 years.
*Selection criteria: Fully described in section 2.3.2. Inclusion criteria: Currently active in the cohort, staying in the study area, child aged 2–3 years. Exclusion criteria: (i) Medical comorbidity (genetic syndrome, neurological disorder, or congenital abnormality); (ii) Gestation <36 weeks; (iii) Low Apgar score (<7 at 5 min); (iv) Neonatal intensive care admission; (v) Maternal use of illicit drugs during pregnancy; (vi) Child HIV infection.

were unable to obtain imaging data for these children. Of the 167 children with at least one successful sequence, 100/167 (60%) slept through the full 5 sequences (T1-MEMPRAGE, resting state fMRI, MRS, DTI and T2-weighted sequences) and 67/167 (40%) slept through between 1 and 4 sequences. In addition to T1-weighted images, 163/167 (98%) children had resting state fMRI, 156/167 (93%) MRS, 143/167 (86%) DTI and 107/167 (64%) T2-weighted images. Unsuccessful scans were primarily due to children not falling asleep which was often compounded by minor illness, for example a cough. There were no associations found between child age, child sex or neurodevelopment and scan success (Table 2).

3.2. Cortical structure and neurocognitive development

146/167 (87.4%) children had an included T1-weighted scan and neurocognitive assessment data available for these analyses (Fig. 1). Children had a mean age of 34.0 months and 57.5% were male (see Table 3). Of note, the proportions of children with developmental performance < -1SD below high income country norms included 37.0% in the cognitive domain and 52.2% in language, which are representative of the developmental outcomes reported across the broader DCHS.

Associations between cortical surface area and thickness and cognitive and language development are shown in Table 4 and Fig. 2. Cortical

Table 2
Association of scan success with sociodemographic factors and developmental outcomes (n = 216).

Variable	Fully successful (5 sequences) (n = 100)	1-4 successful sequences (n = 67)	Unsuccessful (0 sequences) (n = 49)	p-value[a]
Age (months), mean (SD)	34.2 (2.0)	34.5 (1.7)	34.7 (1.8)	0.30
Sex (male)	60 (60.0%)	37 (55.2%)	25 (51.0%)	0.57
Cognitive development				
Composite score, mean (SD)	86.4 (9.3)	86.0 (10.1)	85.1 (8.8)	0.74
Developmental delay < -1SD, n (%)	35 (38.5%)	21 (35.6%)	25 (53.2%)	0.15
Language development				
Composite score, mean (SD)	83.8 (10.1)	85.8 (13.5)	83.4 (10.5)	0.50
Developmental delay < -1SD, n (%)	48 (56.5%)	25 (44.6%)	26 (56.5%)	0.33

[a] Footnote: 1-way ANOVA and Chi-square tests performed to compare the three scan success groups.

Table 3
Sociodemographic and neurodevelopmental characteristics of children with an included T1-weighted scan and neurocognitive assessment (n = 146).

Variable	N (%) or Mean (SD)
Age months	34.0 (1.7)
Sex (male)	84 (57.5%)
Monthly household income (ZAR)	
< R1000 (<~$75)	48 (32.9%)
R1000-R5000 (~$75–375)	86 (58.9%)
>R5000 (>~$375)	12 (8.2%)
Maternal education	
Primary	7 (4.8%)
Secondary	91 (62.3%)
Completed secondary	40 (27.4%)
Any tertiary	8 (5.5%)
Maternal employment status (employed)	41 (28.1%)
SES quartile	
Lowest SES	29 (19.9%)
Low-mod SES	35 (24.0%)
Mod-high SES	45 (30.8%)
High SES	37 (25.3%)
Cognitive development	
Composite score, mean (SD)	86.4 (9.3)
Developmental delay < -1SD, n (%)	54 (37.0%)
Language development	
Composite score, mean (SD)	84.4 (11.5)
Developmental delay < -1SD, n (%)	72 (52.2%)

Footnote: Missing data: Language (n = 8).

morphometry in the frontal lobe showed the strongest association with cognitive development including cortical surface area in the right paracentral region (beta coefficient −0.20 (95% confidence interval [CI] −0.39 to −0.01) and cortical thickness in the left caudal middle frontal region (beta coefficient −0.23 [95% CI −0.38 to −0.07]). Regions-of-interest in the frontal and temporal lobes were associated with language development. Cortical surface area in the left and right fusiform (beta coefficient 0.29 [95% CI 0.07 to 0.50] and 0.26 [95% CI 0.03 to 0.48] respectively), and right lateral orbitofrontal region (beta coefficient 0.27 [95% CI 0.03 to 0.52]) showed positive associations with language development. Overall, thinner cortices were associated with higher language scores. Cortical thickness in regions of the frontal lobe including the left and right medial orbitofrontal regions (beta coefficient −0.21 [95% CI −0.37 to −0.05] and −0.29 [95% CI −0.45 to −0.13] respectively), right lateral orbitofrontal (beta coefficient −0.20 (95% CI −0.35 to −0.04) and right rostral middle frontal areas (beta coefficient −0.20 [95% CI -0.36 to −0.04]) all showed negative associations with language with beta coefficients ≥0.20 (Table 4).

Given the differences between the cohort and neuroimaging subgroup in terms of household income, a further analyses was performed including household income as a confounder (Supplementary Table 3). We show that the effect estimates hold. A sensitivity analysis was also performed excluding children classified as extreme outliers (Supplementary Table 4) which did not substantially change the results.

4. Discussion

Our study demonstrates the feasibility of paediatric multimodal neuroimaging without sedation or anesthesia at 2–3 years in a sub-Saharan African setting, where 66% of children under-5 are at risk of developmental impairment (Black et al., 2017). We also provide preliminary analyses addressing relationships between cortical thickness and surface area with cognitive and language development in this age group. To our knowledge this is the first cohort study in SSA to report the association of neuroimaging with developmental data at this age. The methods used to encourage sleep are low-cost, associated with minimal risk and may be implemented in other LMIC settings, offering a viable alternative to sedation or anesthesia. Given the global focus on early child development (Black et al., 2017; Every Woman Every Child, 2015; McDonald et al., 2016) and the value of MRI in the investigation of brain development, this longitudinal cohort of in-depth structural and functional MRI may be useful in understanding the various factors affecting child brain development in this setting, so complementing work in high income countries (Almli et al., 2007; Walker et al., 2016).

Neuroimaging children has intrinsic challenges due to motion and limited cooperation, particularly in the preschool years (Barkovich et al., 2019). We showed a 77% success rate for scanning children without sedation. Neuroimaging young children during non-sedated sleep has not previously been described in a SSA setting, and our results compare favourably to studies in high income countries (Dean et al., 2014). A recent study of children in Canada aged 2–5 years showed success rates of 72% utilising a mock scanner to prepare for the MRI (Thieba et al., 2018), and a large US study reported two thirds success in children between 0 and 5 years (Almli et al., 2007). Imaging children under the age of 5 years without sedation is logistically challenging and it was not developmentally appropriate to use a mock scanner or audio/visual systems emulating MRI sounds in children under 3 years. However, sedation and anesthesia have safety and ethical limitations (Schmidt et al., 2011), utilise resources, and may not be used on all children (including those with respiratory issues). Furthermore, there have been reports of later developmental and behavioural risks associated with anesthesia exposure in childhood (DiMaggio et al., 2011). We conducted MRI during natural sleep adapting methods that have been shown to be successful in high income countries (Raschle et al., 2012; Jaimes and Gee, 2016) to this setting. Our dedicated team used behavioural and play therapy techniques to maximise the parent and child's comfort and minimise distress, with optimisation of the scanner environment to create a child-friendly space conducive to sleeping. Imaging times were coordinated with child sleeping and nap times. We used melatonin, a neurohormone recognised for its regulation of sleep via the circadian rhythm (Abdelgadir et al., 2018) which has been shown to be safe and effective for inducing sleep in children in our setting without the risk of respiratory compromise or the requirement of specialist monitoring (Ibekwe et al., 2017; Johnson et al., 2002).

Overall, we have shown that imaging during natural sleep is a feasible alternative to sedation that can result in minimal motion and high quality scans, addressing the importance of scanning in LMIC settings (Maxfield

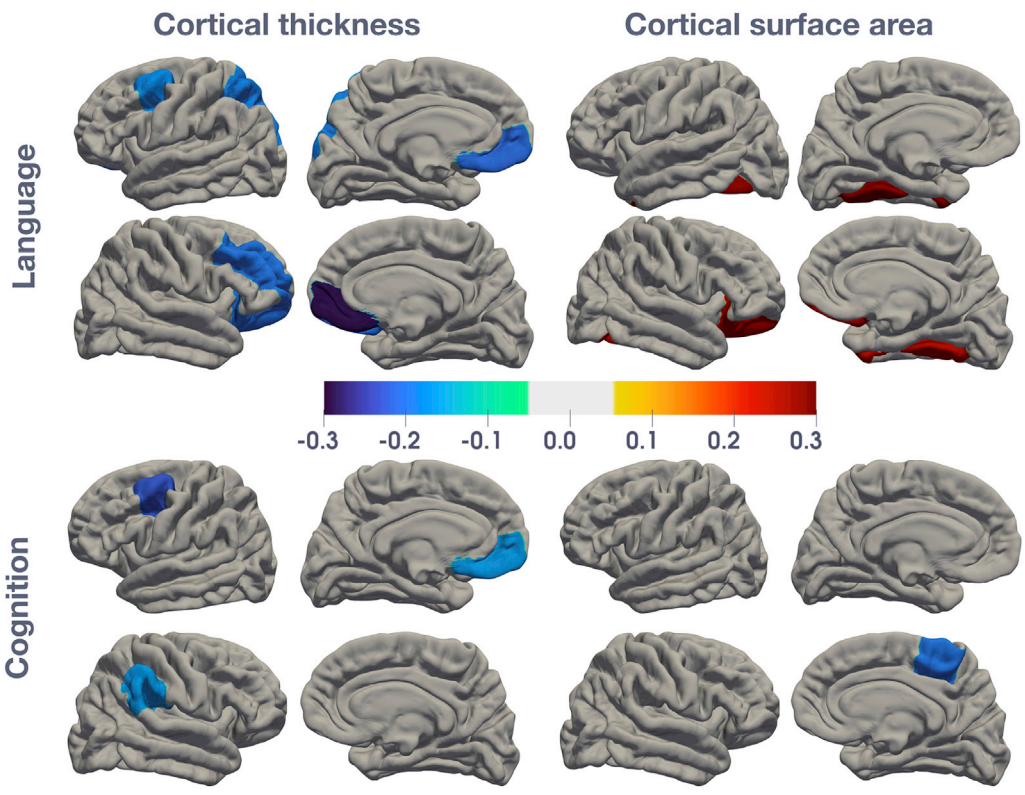

Fig. 2. Statistical maps of effect size (beta coefficients) for the associations between cortical surface area and thickness with cognitive or language development.
Beta coefficients are plotted for each region of interest on a template image. Standardised beta coefficients are calculated from multiple regression models adjusting for child age and sex, and additionally for intracranial volume for surface area measurements. Only significant (uncorrected $p < 0.05$) regions are shown; non-significant regions are coloured in grey. Blue colours represent regions with negative beta coefficients and red represent positive beta coefficients. Please refer to Table 4 for more information.

Table 4
Structural associations (cortical surface area and thickness) of regions-of-interest with cognitive or language development.

					Cognitive development (n = 146)		Language development (n = 138)	
Cortical Surface Area	Lobe	Hemisphere	Mean	SD	Beta coefficient (95% CI)	*P*	Beta coefficient (95% CI)	*P*
Fusiform	Temporal	L	2660	335	0.04 (−0.18 to 0.26)	0.725	0.29 (0.07 to 0.50)**	0.009*
		R	2648	356	0.12 (−0.10 to 0.34)	0.269	0.26 (0.03 to 0.48)**	0.024*
Insula	Temporal	L	1992	204	−0.05 (−0.28 to 0.18)	0.693	0.09 (−0.15 to 0.33)	0.448
		R	1962	241	0.09 (−0.11 to 0.29)	0.393	0.20 (−0.00 to 0.41)**	0.050
Lateral orbitofrontal	Frontal	L	2100	311	0.12 (−0.13 to 0.36)	0.346	0.22 (−0.03 to 0.47)**	0.079
		R	2058	310	0.09 (−0.16 to 0.33)	0.493	0.27 (0.03 to 0.52)**	0.028*
Paracentral	Frontal	L	1192	169	−0.04 (−0.24 to 0.16)	0.699	0.11 (−0.09 to 0.30)	0.294
		R	1315	183	−0.20 (−0.39 to −0.01)**	0.036*	−0.12 (−0.31 to 0.07)	0.215
Cortical Thickness	Lobe	Hemisphere	Mean	SD	Beta coefficient (95% CI)	*P*	Beta coefficient (95% CI)	*P*
Caudal middle frontal	Frontal	L	2.98	0.17	−0.23 (−0.38 to −0.07)**	0.006*	−0.18 (−0.34 to −0.02)	0.027*
		R	2.92	0.18	−0.13 (−0.29 to 0.03)	0.118	−0.11 (−0.28 to 0.05)	0.166
Lateral orbitofrontal	Frontal	L	3.32	0.17	−0.00 (−0.17 to 0.16)	0.956	−0.01 (−0.17 to 0.15)	0.918
		R	3.21	0.17	−0.11 (−0.27 to 0.05)	0.164	−0.20 (−0.35 to −0.04)**	0.014*
Medial orbitofrontal	Frontal	L	3.17	0.21	−0.17 (−0.33 to −0.01)	0.036*	−0.21 (−0.37 to −0.05)**	0.011*
		R	3.18	0.23	−0.16 (−0.32 to 0.01)	0.057	−0.29 (−0.45 to −0.13)**	0.001*
Rostral middle frontal	Frontal	L	3.01	0.13	−0.14 (−0.30 to 0.02)	0.087	−0.10 (−0.26 to 0.06)	0.225
		R	2.91	0.13	−0.12 (−0.28 to 0.04)	0.136	−0.20 (−0.36 to −0.04)**	0.016*
Superior parietal	Parietal	L	2.63	0.13	−0.11 (−0.27 to 0.05)	0.171	−0.19 (−0.35 to −0.03)	0.019*
		R	2.61	0.12	0.03 (−0.14 to 0.20)	0.701	−0.09 (−0.26 to 0.08)	0.318
Supramarginal	Parietal	L	3.03	0.13	−0.00 (−0.16 to 0.16)	0.992	−0.03 (−0.20 to 0.13)	0.696
		R	2.99	0.16	−0.17 (−0.33 to −0.01)	0.035*	−0.15 (−0.31 to 0.01)	0.072

Footnote: Table showing the structural associations between cortical surface area and cortical thickness with cognitive or language development in regions of interest, if either hemisphere had an uncorrected $p < 0.05$. All linear regression models included child age and sex as covariates; associations with surface area also included intracranial volume. The beta (standardised) regression coefficient represents the effect size or expected change in cognitive or language development (in standard deviations) with a one unit standard deviation change in the region-of-interest. Beta coefficients are reported to 2 decimal places. *$p < 0.05$, **absolute beta coefficient$\geq$0.20.

et al., 2019). We found scan success was not associated with child age, sex or neurodevelopmental scores suggesting natural sleep may be used without resulting in selection bias. These approaches may be used to allow effective imaging without sedation in future studies. Furthermore, although this work was conducted in a research context, it has important implications for clinical settings in terms of reducing the number of children needing sedation and anesthesia in routine clinical investigations. In addition, MRI may be performed in locations with limited monitoring and resuscitation facilities (Johnson et al., 2002).

In this study we also explored whether regional variations in brain structure were associated with neurocognitive function at 2–3 years of age. Whereas there are well-defined regional differences in cortical

development trajectories in older children, adolescents and adults, very little is known about cortical maturation at this age, particularly in LMIC (Gilmore et al., 2012; Deoni et al., 2015). We report on both cortical surface area and thickness, and found that cognitive development was most strongly associated with cortical morphometry in frontal regions. This is consistent with studies across all ages where well-defined associations between cognition and frontal areas are reported (Paterson et al., 2006; Shaw et al., 2006; Narr et al., 2007). Studies of older children have identified the frontal lobes, and maturation of the prefrontal cortex in particular, as important for later cognitive tasks, including executive function (Paterson et al., 2006; Ronan et al., 2019). Notably, the frontal areas identified are major heteromodal cortical association regions, suggesting their structural maturation is key to integrated higher-level function.

Language development was positively associated with cortical surface area in temporal and frontal regions, and negatively associated with cortical thickness in multiple frontal areas. Our regional findings are consistent with data from functional studies of younger children which show language associated with activation of the temporal lobe as well as frontal regions (Imada et al., 2006; Dehaene-Lambertz et al., 2002). In adults, there is evidence for a key role of the temporal region (specifically the fusiform gyrus) as well as collateral recruitment of frontal cortex in language (Paterson et al., 2006; Sowell et al., 2003). Other studies have also found cortical thickness negatively correlated to language (and executive function) in childhood, predominantly in the frontal and temporal cortical regions (Shaw et al., 2006; Murner-Lavanchy et al., 2018; Brito et al., 2017; Girault et al., 2019; Porter et al., 2011) Separately, white matter in frontal and temporal cortices has also been linked to receptive and expressive language development (O'Muircheartaigh et al., 2014). The orbitofrontal region showed associations with language for both cortical surface area and thickness. This area of the prefrontal cortex has been identified as important for higher functions later in life, including language-related tasks such as sentence completion (Elliott et al., 2000), and the relationship with language at this age may lay the groundwork for later higher order functions and more complex language development. Our results show associations in bilateral hemispheres consistent with findings from a study measuring white matter and language (Walton et al., 2018), which suggested that preschool children may use an extensive language processing network that becomes more left lateralised from 5 years (Paterson et al., 2006; Weiss-Croft and Baldeweg, 2015; Bates et al., 1997). Overall these exploratory analyses support current evidence of a dynamic interplay between brain structure and function over the first few years of life.

Cortical thickness and surface area are key components of cortical structure and their maturation over the first years is thought to play a critical role in later developmental outcomes (Remer et al., 2017). There are few previous studies of cortical surface area and thickness in this age group. In this study higher neurocognitive development scores were associated with reduced cortical thickness and generally increased surface area in specific regions. The negative associations of cognitive and language development with cortical thickness are consistent with previous reports (Shaw et al., 2006). Cortical thickness increases in infancy until two years (Lyall et al., 2015; Wang et al., 2019) followed by region-specific cortical thinning thereafter (Remer et al., 2017). The decreases in cortical thickness may reflect synaptic pruning or increased myelination (Brito et al., 2017; Natu et al., 2019), and the creation of a more efficient cortical network. Decreases in cortical thickness have been associated with improved cognitive performance, particularly in the frontal regions (Murner-Lavanchy et al., 2018; Wang et al., 2019; Burgaleta et al., 2014). A recent study reported cortical thickness decreases from as early as 1 year in some regions (Remer et al., 2017). Cortical surface area increases at a slower rate than cortical thickness and peaks later around nine years (Wierenga et al., 2018; Gilmore et al., 2018) consistent with the associations we found at aged 2–3 years being mainly positive. Potentially one explanation for the different direction of association in the cognitive analysis may be areas peaking earlier in development.

There are methodological considerations associated with this type of imaging research in young children. Incidental findings need to be managed responsibly (Jansen et al., 2017). We only report two incidental findings of clinical significance and the appropriate referral pathways were in place. In the structure-cognition analyses, overall, effects were modest but similar in magnitude to other studies examining brain structure and cognitive function (Gilmore et al., 2018; Shaw et al., 2006; Narr et al., 2007; Ronan et al., 2019) Longitudinal studies and larger sample sizes are likely needed to show strong associations given the extensive brain development over the first few years of life and differences in individual brain trajectories. One study also found that the dynamic change in cortical structure was more closely related to intelligence than a static measure (Shaw et al., 2006). Furthermore, as multiple regions are responsible for neurocognitive function, investigating other modalities, including diffusion tensor imaging and functional MRI, will be useful to inform the network of regions and connections at the brain-cognition interface.

This is the first study to investigate both cortical structure and thickness in preschool South African children and the relationship with cognitive function. Strengths of this study include the comprehensive neuroimaging and clinical assessments and large sample size. This study also has limitations. The neuroimaging sub-group inclusion criteria were based upon the data collected up to the point of sample selection, and the accuracy of gestational age data was limited by the tools available. Secondly, the results are exploratory and do not allow causal inference. We have reported uncorrected p-values throughout due to the approach of this analysis and therefore our results must be interpreted as exploratory and require replication in other studies. We therefore focus on effect sizes throughout and discuss the strength of associations. Thirdly, we adjusted for child age and sex but not for other potential sociodemographic confounding factors in this proof-of-concept analysis, and further work is needed to explore these. Overall, our sample was representative of the wider DCHS parent study in terms of sociodemographic variables, differing only in household income which did not substantially affect results in our sensitivity analyses and there is little variability across the DCHS. Brito and colleagues found socioeconomic disadvantage may exaggerate links between brain structure and cognitive development (Brito et al., 2017). In that study, negative associations between cortical thickness and cognition were more robust for children from lower socioeconomic status homes, similar to the majority of families in the DCHS cohort. We therefore feel the results are generalisable to the wider population.

From a public health perspective, given the global burden of neurodevelopmental impairment in children is greatest in LMIC, neuroimaging research in these contexts is necessary to map brain development, cognition and early origins of disease (Dubois et al., 2014; Morita et al., 2016). Overall, these findings are consistent with our *a priori* hypotheses that frontal regions show (unadjusted within the selected ROIs) associations of thickness and surface area with cognitive development, and frontal and temporal areas with language. This lays the foundation for further work to explore the genetic and environmental influences on child development (Gilmore et al., 2018). Future hypotheses-driven analyses will examine the impact of environmental factors on brain structure and function in the DCHS cohort (Donald et al., 2015; Tran et al., 2016).

5. Conclusions

In conclusion, we demonstrate a successful methodological approach to neuroimaging without sedation under the age of 5 years in sub-Saharan Africa. Given the importance of early child development and use of MRI to investigate developmental delay, further studies may utilise these approaches to allow effective imaging during natural sleep. In this proof-of-concept analysis we also demonstrate regional associations between cortical surface area and thickness with cognitive and language development at 2–3 years, similar to brain-cognition studies from high

income settings. These provide future directions for understanding early child development and to examine the impact of socioenvironmental factors in this context.

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Declaration of competing interest

The authors report no conflicts of interest.

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Catherine J. Wedderburn: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Sivenesi Subramoney:** Data curation, Investigation, Writing - review & editing. **Shunmay Yeung:** Supervision, Writing - review & editing. **Jean-Paul Fouche:** Software, Formal analysis, Writing - review & editing. **Shantanu H. Joshi:** Software, Visualization, Writing - review & editing. **Katherine L. Narr:** Conceptualization, Methodology, Resources, Writing - review & editing. **Andrea M. Rehman:** Formal analysis, Writing - review & editing. **Annerine Roos:** Investigation, Writing - review & editing. **Jonathan Ipser:** Methodology, Software, Validation. **Frances C. Robertson:** Resources, Writing - review & editing. **Nynke A. Groenewold:** Validation, Writing - review & editing. **Diana M. Gibb:** Supervision, Writing - review & editing. **Heather J. Zar:** Conceptualization, Methodology, Resources, Writing - review & editing. **Dan J. Stein:** Conceptualization, Methodology, Resources, Writing - review & editing. **Kirsten A. Donald:** Conceptualization, Methodology, Resources, Investigation, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

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8

Chapter 8: Early Structural Brain Development in Infants Exposed to HIV and Antiretroviral Drugs *in Utero* in a South African Birth Cohort

(Research paper)

Early structural brain development in infants exposed to HIV and antiretroviral therapy *in utero* in a South African birth cohort

Summary

Chapter 8 presents the neuroimaging results for the infants at 2-6 weeks of age, representing the fifth research paper titled: 'Early structural brain development in infants exposed to HIV and antiretroviral therapy *in utero* in a South African birth cohort'. This paper addresses objective 2 by examining the differences in brain structure between HEU and HU infants at the first imaging timepoint. Due to the rapid brain maturation and limited myelination at this age, the findings focus on total grey matter and subcortical volumes. The paper also explores the association of maternal immunosuppression, as measured by CD4 in pregnancy, and timing of maternal ART initiation, with infant brain volumes (secondary objective).

Smaller total grey matter volumes and caudate nuclei (part of the basal ganglia) were found in infants who were HEU compared to HU aged 2-6 weeks. Lower maternal CD4 cell count was associated with reduced grey matter volumes with a dose-response, whereas maternal ART initiation in pregnancy versus pre-pregnancy was not. Overall, these findings suggest that antenatal HIV exposure may impact early brain development, with structural differences detectable early in postnatal life indicating that changes may occur *in utero*. Further, the results suggest that improved maternal HIV management in pregnancy and prevention of immunocompromise may have the potential to optimise child neurodevelopmental outcomes.

My role included quality control and image processing of all the infant structural scans under the supervision of Dr Nynke Groenewold (imaging expert). This involved visually inspecting the scans for any anomalies or motion artefacts pre-processing and for errors in normalisation and segmentation post-processing; processing using Statistical Parametric Mapping (SPM) software; and outlier detection using ENIGMA pipelines. For this paper, I designed and conducted the data analysis with advice from A/Prof Andrea Rehman (statistician). I wrote the first manuscript draft and incorporated comments and edits from co-authors and peer reviewers.

List of Figures

List of Tables

Supplementary material

The supplementary material as detailed in the published article is available at https://doi.org/10.1002/jia2.25863 and listed in Appendix VIII.

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Thesis Title	Neurodevelopmental effects of HIV exposure: a prospective neuroimaging study of uninfected children born to mothers living with HIV		
Primary Supervisor	Professor Shunmay Yeung		

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RESEARCH ARTICLE

Early structural brain development in infants exposed to HIV and antiretroviral therapy *in utero* in a South African birth cohort

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Abstract

Introduction: There is a growing population of children who are HIV-exposed and uninfected (HEU) with the successful expansion of antiretroviral therapy (ART) use in pregnancy. Children who are HEU are at risk of delayed neurodevelopment; however, there is limited research on early brain growth and maturation. We aimed to investigate the effects of *in utero* exposure to HIV/ART on brain structure of infants who are HEU compared to HIV-unexposed (HU).

Methods: Magnetic resonance imaging using a T2-weighted sequence was undertaken in a subgroup of infants aged 2–6 weeks enrolled in the Drakenstein Child Health Study birth cohort, South Africa, between 2012 and 2015. Mother–child pairs received antenatal and postnatal HIV testing and ART per local guidelines. We compared subcortical and total grey matter volumes between HEU and HU groups using multivariable linear regression adjusting for infant age, sex, intracranial volume and socio-economic variables. We further assessed associations between brain volumes with maternal CD4 cell count and ART exposure.

Results: One hundred forty-six infants (40 HEU; 106 HU) with high-resolution images were included in this analysis (mean age 3 weeks; 50.7% male). All infants who were HEU were exposed to ART (88% maternal triple ART). Infants who were HEU had smaller caudate volumes bilaterally (5.4% reduction, $p < 0.05$) compared to HU infants. There were no group differences in other subcortical volumes (all $p > 0.2$). Total grey matter volume was also reduced in infants who were HEU (2.1% reduction, $p < 0.05$). Exploratory analyses showed that low maternal CD4 cell count (<350 cells/mm^3) was associated with decreased infant grey matter volumes. There was no relationship between timing of ART exposure and grey matter volumes.

Conclusions: Lower caudate and total grey matter volumes were found in infants who were HEU compared to HU in the first weeks of life, and maternal immunosuppression was associated with reduced volumes. These findings suggest that antenatal HIV exposure may impact early structural brain development and improved antenatal HIV management may have the potential to optimize neurodevelopmental outcomes of children who are HEU.

Keywords: HIV exposure; antiretroviral therapy; magnetic resonance imaging; brain structure; newborn infant; child development

Additional information may be found under the Supporting Information tab of this article.

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1 | INTRODUCTION

Over 1.3 million women living with HIV give birth each year [1,2]. With effective antiretroviral therapy (ART), the number of infants infected with HIV has reduced to ~150,000, but paralleled by a growing global population of children who are HIV-exposed and uninfected (HEU) [2]. Infants who are HEU have been found to have higher mortality and morbidity compared to HIV-unexposed (HU) infants [3–7]. Some studies have also suggested that exposure to specific antiretrovirals may be associated with poor pregnancy [6] and neurologic [8,9] outcomes, while others have not [10,11]. Further, cohort studies have reported delayed early neurodevelopment in children who are HEU [12,13], although findings are inconsistent and neurobiological mechanisms are not yet understood [14].

Brain growth and maturation occur rapidly in infancy [15] and form the basis of later cognitive and behavioural outcomes [16]. Advances in neuroimaging allow greater understanding of factors affecting early brain maturation and objective measures of neurodevelopment [17]. While many studies of children with HIV infection have found differences in neuroanatomy [18,19], magnetic resonance imaging (MRI) studies examining brain development in children who are HEU are scarce. Microstructural differences between children who are HEU and HU have been described using diffusion tensor imaging (DTI) in small sample sizes (HEU group <20) [20–23]. Yet, despite the potential use of structural MRI, only one retrospective French study has examined brain morphology in young children who were HEU (mean age 26 months) presenting with neurologic symptoms. Of those children, 45% had mitochondrial dysfunction, and 50% of images showed atypical anatomy, including basal ganglia abnormalities and volume loss [24]. However, children in this study were symptomatic at baseline and predominantly exposed to zidovudine; therefore, the findings may not be generalizable.

Sub-Saharan Africa (SSA) has the highest burden of both HIV and developmental delay globally [1,25]. Given the relationship between brain structure and function, it is critical to understand the effects of antenatal HIV/ART exposure on brain maturation in this context [17]. The South African Drakenstein Child Health Study (DCHS) birth cohort was established to investigate the early-life determinants of child health and development [26,27]. This study is set in an African community with high levels of poverty and other socio-environmental risk factors [28]. A single report from the DCHS has investigated neonates who are HIV-exposed using DTI, and found differences in white matter microstructure at this age [21]. Volumetric brain growth in neonates is driven by grey matter development [15]. Prior studies have shown that grey matter may be affected by *in utero factors*, including maternal substance use [29] and child HIV infection [30], yet, to our knowledge, no published studies have examined the effects of antenatal HIV/ART exposure without infection on neonatal grey matter morphometry. Therefore, the aim of this study was to investigate the effects of *in utero* HIV and ART exposure on the neuroanatomy of newborn infants who are HEU compared to HU. We hypothesized that infants who are HEU would have altered grey matter volumes compared to HU infants.

2 | METHODS

2.1 | Participants

We conducted a prospective neuroimaging study of neonates nested within the DCHS, an observational population-based birth cohort in the Western Cape of South Africa [26,27]. Pregnant women were recruited into the DCHS from two public sector primary healthcare clinics, Mbekweni (serving a predominantly black African community) and TC Newman (serving a mixed ancestry community). Mothers were enrolled into the DCHS during their second trimester if they were at least 18 years old, between 20 and 28 weeks' gestation and intended to remain in the area [26,27].

Between 2012 and 2015, 1143 infants were born to 1137 mothers (Figure 1). Antenatal maternal HIV prevalence was 21%, but only two infants were HIV infected [28]. After birth, a sub-group of 236 newborn infants were invited for neuroimaging (20.6%) [31]. Infants were included if they were aged between 2 and 6 weeks and excluded if they had: (1) medical comorbidities, including a genetic syndrome, neurological disorder or congenital abnormality; (2) history of prematurity (gestation <36 weeks); (3) low Apgar score (<7 at 5 minutes); (4) neonatal intensive care admission; (5) maternal use of illicit drugs during pregnancy; (6) MRI contraindications; or (7) infant HIV infection [29]. Convenience sampling was used to select from eligible infants. In this high-risk population, we paid particular attention to other known risk factors associated with adverse neurodevelopment, including maternal depression and alcohol use, and account for these in sensitivity analyses.

2.2 | Ethical considerations

The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009, 525/2012 and 044/2017) and the Western Cape Department of Provincial Health Research Committee. Written informed consent by mothers was given at enrolment and at the start of each neuroimaging session.

2.3 | Procedures

2.3.1 | HIV/ART data

All women underwent routine HIV testing in pregnancy as per the Western Cape prevention of mother-to-child transmission of HIV (PMTCT) guidelines [32,33]. Mothers who tested negative were retested throughout pregnancy and the postpartum period every 3 months to detect seroconversion. Mothers living with HIV were enrolled into the Provincial PMTCT programme and initiated on ART per guidelines at the time. Before May 2013, the first-line regimen depended on maternal clinical and immunological status consisting of triple ART or zidovudine from 14 weeks' gestation; from May 2013, universal triple ART (commonly tenofovir, emtricitabine plus efavirenz) was given to all pregnant women for life. Infants who were HEU received prophylaxis of nevirapine with or without zidovudine. Additional data on maternal CD4 and viral load during pregnancy were obtained using the online National Health Laboratory Service. HIV-exposed infants were tested for HIV using PCR at 6 weeks of age per the guidelines; all HEU infants were confirmed to have a negative HIV test result. HU infants were born to mothers with confirmed HIV negative status.

2.3.2 | Socio-demographic variables

Socio-demographic data were collected using validated questionnaires during interviews with trained study staff [34]. Birth data were abstracted from hospital records. Child gestational age at delivery was calculated to define prematurity based on antenatal ultrasound if available, otherwise using symphysis-fundal height or maternal report of last

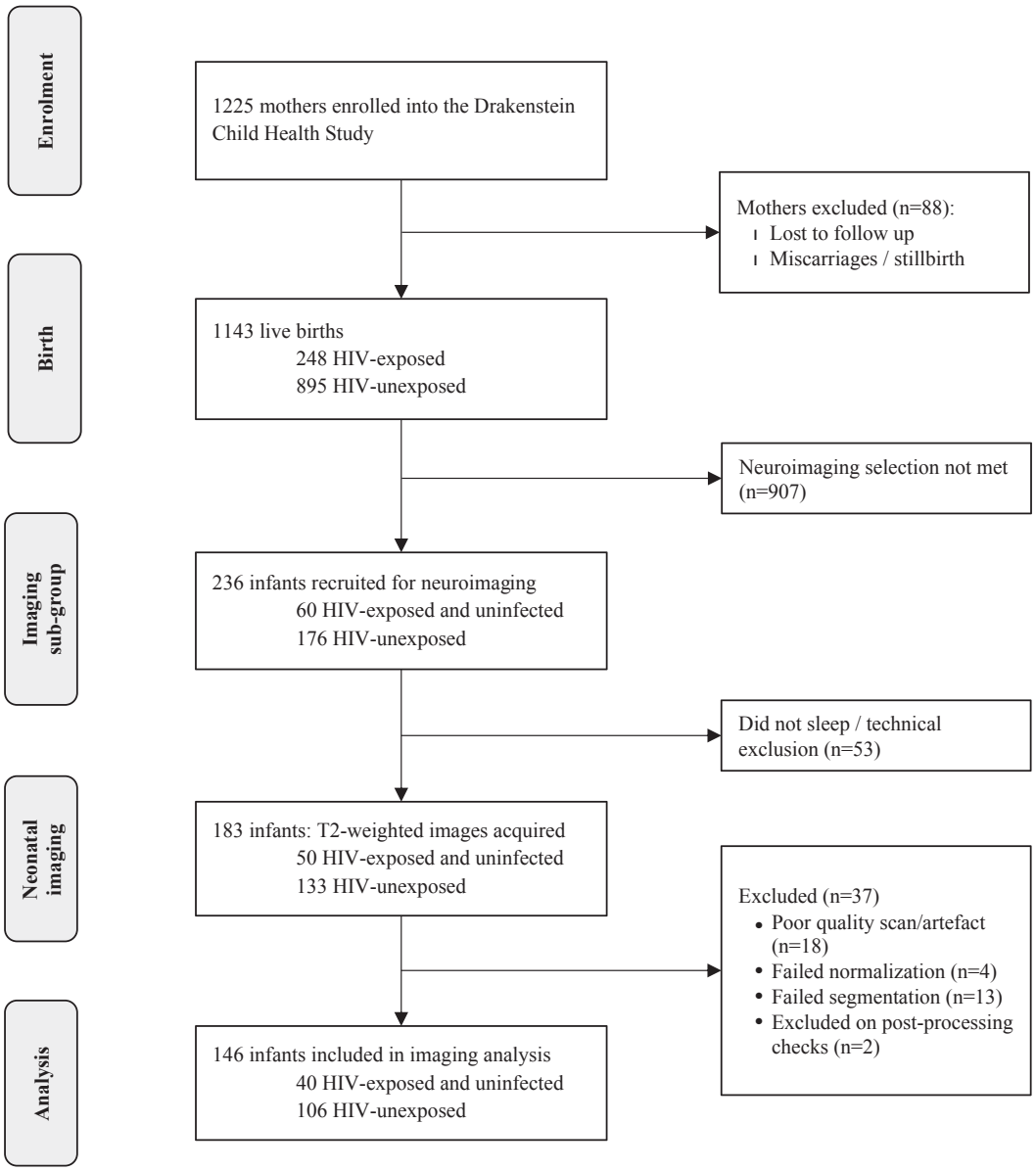

Figure 1. Drakenstein Child Health Study cohort flow chart of children with neuroimaging.

menstrual period [28]. Maternal data on alcohol use, smoking and depression were collected at antenatal visits and birth [27].

2.3.3 | *Cytomegalovirus testing*

Nasopharyngeal swabs were collected from a subgroup of infants (n = 111) between birth and the MRI scan as part of the DCHS protocol. Cytomegalovirus (CMV) DNA was measured using quantitative multiplex real-time PCR on FTDResp33 (Fast-track Diagnostics, Luxembourg). These methods are described in full elsewhere [35].

2.3.4 | *Neuroimaging acquisition*

T2-weighted MR images [36] were acquired at the Cape Universities Brain Imaging Centre, at Tygerberg Hospital from September 2012 to September 2015 using a Siemens Magnetom 3T Allegra MRI scanner (Erlangen, Germany). Neuroimaging was conducted during natural sleep without sedation [21] (Supporting Information).

2.3.5 | *Neuroimaging processing*

Neuroimaging data were available for 183 neonates (50 HEU; 133 HU). T2-weighted images were processed using Statistical Parametric Mapping (SPM8) software blinded to child

HIV/ART exposure status, to generate measures of brain structure. Images were segmented and total grey matter and subcortical regions-of-interest (thalamus, caudate, putamen, pallidum, amygdala and hippocampus) were defined per the automated anatomical labelling atlas [37] adapted to a neonate grey matter template in Montreal Neurological Institute standard space [38]. Volumes were extracted from segmented grey matter images bilaterally. Quality control was performed by two researchers to identify any scan artefacts and errors of segmentation. Outlier detection was performed using the ENIGMA protocol [39]. Any regions marked as a statistical outlier were re-inspected to confirm accurate segmentation.

The mean of the left and right hemispheres was calculated for each subcortical structure. Intracranial volume (ICV) was calculated as total grey matter, white matter and cerebrospinal fluid.

2.4 | Statistical analysis

Socio-demographic and clinical characteristics were compared between HEU and HU groups using unpaired *t*-tests or Mann–Whitney U tests (continuous variables), and chi-squared tests or Fisher's exact tests (categorical variables). Group differences in subcortical and total grey matter volumes were assessed using linear regression. In each model, HIV exposure was considered as the independent variable and grey matter volume as the dependent variable. Confounders were selected *a priori*. Minimally adjusted models included infant age at scan, infant sex and ICV (to account for variability in head size). Fully adjusted models were created using a Directed Acyclic Graph [4,12] to determine the minimal adjustment set of variables and additionally included maternal education, household income and maternal age. Standardized effect sizes were calculated using Cohen's d. Residuals were checked for normality using quantile–quantile plots and homogeneity of variance using scatterplots. Percentage differences (% absolute difference in volume between HEU and HU infants relative to the mean volume in the control group for the particular structure) were also calculated.

Among infants who were HEU, the effects of maternal disease severity (measured through CD4 cell count) and maternal ART were explored through secondary regression analyses. CD4 cell counts were binned into three categories, <350, 350–500 and >500, per previous guidelines [33]. For ART exposure, analyses were performed firstly comparing infants exposed to maternal triple ART versus zidovudine only; and secondly, examining timing of initial ART exposure, comparing maternal ART initiation *preconception* versus *during pregnancy* given reports of adverse outcomes associated with ART exposure at conception [40]. We also conducted analyses limiting to infants exposed to the same WHO-recommended first-line regimen that most women received (tenofovir + emtricitabine/lamivudine + efavirenz) to allow us to examine the timing of initial ART exposure without the confounding effects of different antiretroviral drugs.

Finally, sensitivity analyses were performed to assess the robustness of the results by: (1) excluding imaging outliers; (2) restricting to neonates ≤28 days old at imaging; (3) restricting to infants from one clinic, given site differences and that site was closely correlated to HIV status; (4) assessing additional potential confounders, including maternal prenatal alcohol use, smoking and depression, that may be on the causal pathway; and (5) excluding infants who tested CMV positive at any point (Supporting Information).

Data analysis was performed using STATA (StataCorp Inc, College Station, TX, USA) version 14.2. $p < 0.05$ was used as the threshold of statistical significance. The mean volume of each bilateral subcortical structure was used to minimize the number of comparisons; however, where a region was associated with HIV exposure status ($p < 0.05$), we conducted exploratory analyses stratified by hemisphere. We also performed additional correction for multiple comparisons across the subcortical regional models ($n = 6$) using the standard false discovery rate method with a false-positive rate of 5% ($q = 0.05$) [41].

3 | RESULTS

3.1 | Characteristics of infants who are HEU and HU

A total of 146 infants (40 HEU; 106 HU) had T2-weighted structural MRIs that passed quality control (Figure 1). HEU and HU groups were comparable in terms of age at scan, sex and socio-economic factors, although mothers with HIV were older. Median age at scanning was 21 days (IQR 18–26) and 51% were male. Higher prevalence of prenatal smoking, alcohol use and depression occurred in the HU group (Table 1). All infants who were HEU were ART exposed (88% to maternal triple ART; 38% exposed from conception). Median maternal CD4 cell count during pregnancy was 423 cells/mm^3 (IQR 286–594). The imaging subsample was largely representative of the population included in the wider study (Table S1).

3.2 | Subcortical volumes

Infants who were HEU had smaller caudate volumes in comparison to HU (1785 vs. 1886 mm^3, $p = 0.0009$, Cohen's d effect size −0.58 [95% CI −0.95 to −0.21]) independent of infant age, sex and ICV (Table 2 and Figure 2). When adjusting for socio-economic confounders (maternal age, education and household income), the associations held with a similar effect size −0.57 (−0.94 to −0.20) and remained significant after multiple comparison correction (Table 2). This effect was evident bilaterally (left hemisphere $p = 0.015$, effect size: −0.45 [−0.82 to −0.09]; right hemisphere $p = 0.0002$, effect size: −0.62 [−1.00 to −0.25]) with volumetric reductions of 5.4% overall, 4.1% left hemisphere and 6.6% right hemisphere. There were no group differences in other subcortical regions (all $p > 0.2$).

3.3 | Total grey matter

Total grey matter volume was 2.1% smaller in infants who were HEU (233,713 vs. 238,676 mm^3, $p = 0.035$, minimally adjusted Cohen's d effect size: −0.33 [−0.69 to 0.04]) compared to HU infants (Table 2 and Figure 3). There remained strong evidence for association after further adjusting for potential socio-economic confounders (Table 2).

Table 1. Socio-demographic and clinical characteristics of infants according to HIV exposure

Variable	Total (*n* = 146)	HEU (*n* = 40)	HU (*n* = 106)	*p*-value
Socio-demographic characteristics				
Child age at scan, days	21 (18–26)	23 (19–28)	21 (18–26)	0.326
Child post-conceptual age at scan, days	298 (291–307)	298 (286–309)	298 (293–306)	0.714
Sex				
Female	72 (49.3%)	18 (45.0%)	54 (50.9%)	0.522
Male	74 (50.7%)	22 (55.0%)	52 (49.1%)	
Site				
TC Newman	73 (50.0%)	1 (2.5%)	72 (67.9%)	<0.001*
Mbekweni	73 (50.0%)	39 (97.5%)	34 (32.1%)	
Monthly household income (ZAR)				
<R1000 (<~$75)	49 (33.6%)	13 (32.5%)	36 (34.0%)	0.513
R1000–R5000 (~$75–375)	75 (51.4%)	23 (57.5%)	52 (49.1%)	
>R5000 (>~$375)	22 (15.1%)	4 (10.0%)	18 (17.0%)	
Maternal education				
Any primary	7 (4.8%)	2 (5.0%)	5 (4.7%)	0.915
Any secondary	73 (50.0%)	20 (50.0%)	53 (50.0%)	
Completed secondary	58 (39.7%)	15 (37.5%)	43 (40.6%)	
Any tertiary	8 (5.5%)	3 (7.5%)	5 (4.7%)	
Maternal employment status (employed)	40 (27.4%)	11 (27.5%)	29 (27.4%)	0.986
Relationship status (married/cohabitating)	63 (43.5%)	16 (40.0%)	47 (44.8%)	0.605
Maternal age at birth, years	26.9 (22.0–31.6)	29.4 (24.6–33.2)	26.1 (21.7–30.0)	0.017*
Gestational age at birth, weeks	39 (38–40)	39 (38–40)	39 (38–40)	0.390
Birthweight, g	3157 (462)	3110 (443)	3174 (469)	0.462
Birth head circumference, cm	33.8 (1.7)	33.8 (1.8)	33.8 (1.7)	0.957
Maternal smoking during pregnancy				
Active	45 (31.0%)	6 (15.0%)	39 (37.1%)	
Passive	61 (42.1%)	22 (55.0%)	39 (37.1%)	
Non-smoker	39 (26.9%)	12 (30.0%)	27 (25.7%)	0.031*
Maternal alcohol use during pregnancy	25 (17.9%)	2 (5.6%)	23 (22.1%)	0.025*
Maternal depression	44 (31.4%)	6 (16.7%)	38 (36.5%)	0.027*
Maternal hospitalization	8 (5.5%)	1 (2.5%)	7 (6.6.%)	0.446
Neuroanatomical variables				
Total intracranial volume (mm^3)	425,903 (4434)	425,352 (5237)	426,111 (4100)	0.674
Maternal and child HIV variables				
Maternal HIV diagnosis time-point				
Before pregnancy		27 (71.1%)		
During pregnancy		11 (29.0%)		
Maternal CD4 cell count				
Median (interquartile range) (cells/mm^3)		423 (286–594)		
<350 cells/mm^3		13 (39.4%)		
350–500 cells/mm^3		8 (24.2%)		
>500 cells/mm^3		12 (36.4%)		
Maternal viral load (VL) in pregnancy				
Lower than detectable limit (<40 copies/ml)		19 (73.1%)		
VL detectable (≥40–1000 copies/ml)		5 (19.2%)		
Virally unsuppressed (≥1000 copies/ml)		2 (7.7%)		
Antiretroviral drug initiation				
Before conception		15 (37.5%)		
During pregnancy		25 (62.5%)		

(*Continued*)

Table 1. Continued

Variable	Total (n = 146)	HEU (n = 40)	HU (n = 106)	p-value
Antiretroviral regimen during pregnancy				
Monotherapy with AZT (zidovudine)		5 (12.5%)		
2 NRTIs + NNRTI (first line)		34 (85.0%)		
2 NRTIs + PI (second line)		1 (2.5%)		
Infant prophylaxis				
NVP [nevirapine] alone		35 (87.5%)		
NVP + AZT		5 (12.5%)		

Note: Data are presented as *n/N* (%), mean (SD) or median (IQR). *p*-values represent group-comparison with two-sided unpaired *t*-tests or Mann–Whitney U tests (continuous variables), or chi-squared or Fisher's exact test if any n <5 (categorical variables) as appropriate.
Abbreviations: AZT, zidovudine; HEU, HIV-exposed and uninfected; HU, HIV-unexposed; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; VL, viral load.
*p < 0.05. Percentages are cited among those with non-missing values. Missing data: relationship status (n = 1); birthweight (n = 1); smoking (n = 1); alcohol (n = 6); depression (n = 6); HIV diagnosis time-point (n = 2); CD4 (n = 7); and viral load (n = 14). Specific variables were assessed as follows: (1) maternal smoking was measured by urine cotinine levels taken antenatally/birth; (2) maternal alcohol use was assessed and quantified using the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) and retrospectively collected data on moderate-severe alcohol use in pregnancy forming a dichotomous measure; (3) maternal depression in pregnancy was measured using the Edinburgh postnatal depression scale (EPDS); (4) the lowest maternal CD4 during pregnancy was used to reflect maternal immunosuppression in pregnancy; (5) maternal viral load was categorized into <40 copies/ml as lower than the detectable limit, ≥40 to <1000 copies/ml as detectable and ≥1000 copies/ml as unsuppressed. Where there was more than one result, the highest viral load during pregnancy was taken; (6) of those mothers initiating ART during pregnancy, timing of initiation: 6 (24%) first trimester, 14 (56%) second/third trimester, 5 (20%) unknown; (7) first-line triple therapy: a non-nucleoside reverse-transcriptase inhibitor backbone and two nucleoside reverse transcriptase inhibitors, most commonly efavirenz with tenofovir and emtricitabine as a fixed-dose combination (n = 31, 77.5%); however, some mothers received nevirapine-based treatment (n = 3).

3.4 | Sensitivity analyses

We confirmed the findings by conducting the analyses excluding statistical outliers (Table S2) and restricting firstly to those infants who were ≤28 days of age (Table S3); and secondly to those infants who were attending Mbekweni clinic, where the majority of infants who are HEU were attending (Table S4), resulting in associations of a similar magnitude and direction. Our findings persisted after adjusting for relevant psychosocial variables that differed between groups, including prenatal exposure to maternal depression, smoking and alcohol that were higher in the control HU group (Tables S5–S7). No child had evidence of congenital CMV disease. A low prevalence of infant CMV infection was detected on nasopharyngeal swabs taken prior to the scan date, with one HEU and one HU infant testing positive (no group difference, p = 0.570). Excluding these infants from the analysis did not meaningfully change the associations or effect sizes (Table S8).

3.5 | Association with maternal immune function and ART

In exploratory analyses, we found maternal CD4 predicted infant total grey matter volumes (Figure 3 and Table S9). Our results suggest that there is a dose–response association with lower maternal CD4 associated with smaller infant volumes, and infants who were HEU born to mothers with CD4 cell count <350 cells/mm^3 had the lowest volumes. We found no relationship between maternal ART regimen or timing of initiation and infant global and regional brain volumes (p > 0.05) (Table S10). In analyses restricted to infants who were HEU exposed to the same first-line triple ART, there was also no effect of ART initiation timing. In this sub-group, the relationship between maternal immunological compromise and decreased infant grey matter volumes was strengthened for both caudate and total grey matter (Table 3).

4 | DISCUSSION

In this study, we used MRI to examine the impact of HIV/ART exposure on brain structure. Overall, infants who were HEU had smaller total grey matter and subcortical caudate volumes bilaterally compared to infants who were HU, independent of ICV and socio-economic factors. In exploratory analyses, maternal immunosuppression was associated with reduced volumes. Our results suggest that *in utero* exposure to HIV may affect brain maturation of specific regions, and changes in brain structure in infants who are HEU can be detected at a very early stage of neurodevelopment. This concurs with recent literature on early growth trajectories, suggesting prenatal origins of developmental abnormalities in children who are HEU [42,43].

We found that the caudate, a major nucleus of the basal ganglia and integral part of the cortico-striatal network, was smaller in volume in infants who were HEU compared to HU. Other subcortical regions were not affected. Although there are no prior publications on basal ganglia volume in children who are HEU, basal ganglia abnormalities have been frequently reported in adults [44] and children with HIV [19,30], despite ART. Furthermore, magnetic resonance spectroscopy studies have found altered basal ganglia metabolite levels in school-aged children who are HEU at 9 years [45] (although not 11 years [46]), and who are living with HIV

Table 2. Adjusted mean differences in total and regional grey matter volumes between infants who are HIV-exposed and uninfected compared to HIV-unexposed

Brain volumes	HEU Mean (SD) (n = 40)	HU Mean (SD) (n = 106)	Minimally adjusted[a] difference (95% CI)	p-value	Effect size, Cohen's d (95% CI)	Fully adjusted[b] difference (95% CI)	p-value	Effect size, Cohen's d (95% CI)
Global								
Total grey matter	233,713 (15,402)	238,676 (12,127)	−4385 (−8455 to −315)	0.035*	−0.33 (−0.69 to 0.04)	−4352 (−8525 to −179)	0.041*	−0.33 (−0.69 to 0.04)
Subcortical regions								
Thalamus	3205 (113)	3209 (105)	3.76 (−34.03 to 41.54)	0.845	0.04 (−0.33 to 0.40)	6.27 (−32.50 to 45.04)	0.750	0.06 (−0.31 to 0.42)
Caudate	1785 (155)	1886 (158)	−97.54 (−154.39 to −40.68)	<0.001*,§	−0.58 (−0.95 to −0.21)	−96.67 (−154.33 to −39.00)	0.001*,§	−0.57 (−0.94 to −0.20)
Putamen	2830 (86)	2839 (85)	−5.60 (−35.57 to 24.36)	0.712	−0.07 (−0.43 to 0.30)	−3.91 (−34.85 to 27.03)	0.803	−0.05 (−0.41 to 0.32)
Pallidum	711 (33)	712 (30)	−0.32 (−11.15 to 10.50)	0.953	−0.01 (−0.37 to 0.35)	−0.43 (−11.62 to 10.76)	0.940	−0.01 (−0.38 to 0.35)
Hippocampus	1563 (162)	1601 (128)	−24.42 (−68.40 to 19.56)	0.274	−0.18 (−0.54 to 0.19)	−25.28 (−70.64 to 20.07)	0.272	−0.18 (−0.55 to 0.18)
Amygdala	535 (32)	541 (21)	−4.05 (−12.14 to 4.04)	0.324	−0.16 (−0.53 to 0.20)	−3.76 (−12.16 to 4.63)	0.377	−0.15 (−0.52 to 0.21)

Note: Subcortical volumes (mean of left and right hemispheres) in mm^3, mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p values and effect sizes for associations between brain volumes and HIV exposure. Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. Residuals were assessed for each model using quantile–quantile plots and were normally distributed. Linear regression models shown where negative estimates indicate that HIV exposure is associated with lower volumes in that region.

Abbreviations: CI, confidence interval; HEU, HIV-exposed and uninfected; HU, HIV-unexposed.

[a]Minimally adjusted models included child age at scan, child sex and intracranial volume.

[b]Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income and maternal age.

*Uncorrected $p < 0{\cdot}05$.

§p-Values survive multiple comparison correction using the false discovery rate across the six subcortical regions, which generated a corrected overall p-value of 0.0083.

For regions with a significant difference between HEU and HU infants, the percentage volume difference was calculated as the absolute mean difference expressed as a percentage of the mean volume of the HU control group: total grey matter = 2.1% reduction; total caudate = 5.4% reduction. Post hoc the caudate was assessed bilaterally: left hemisphere: 4.1% reduction, fully adjusted Cohen's d effect size: −0.45 (95% CI −0.82 to −0.09). Right hemisphere: 6.6% reduction, fully adjusted Cohen's d effect size: −0.62 (95% CI −1.00 to −0.25).

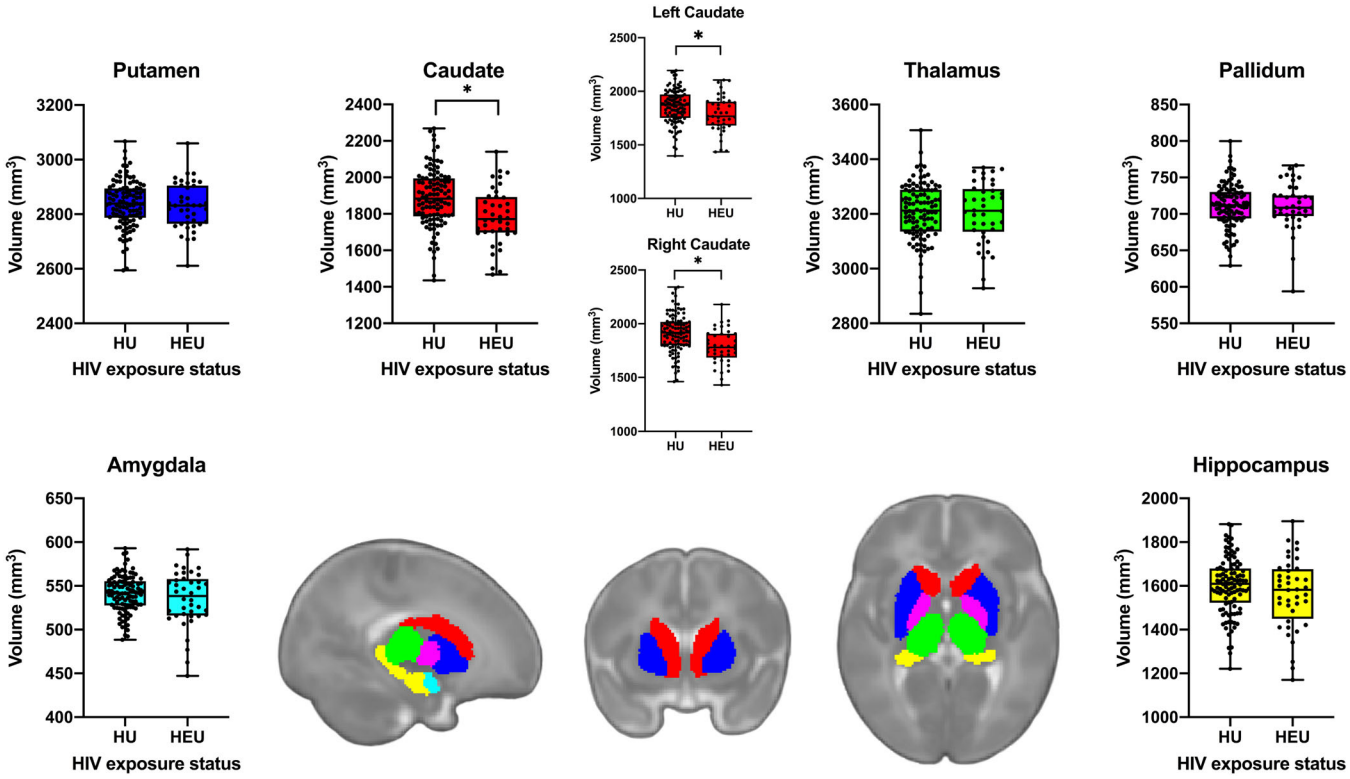

Figure 2. Comparison of subcortical volumes between infants who are HEU and HU. Mean volumes (of left and right hemispheres) for each subcortical region comparing infants who are HEU (n = 40) and HU (n = 106) with an associated atlas of brain regions. *$p < 0.05$ after multiple comparison correction. The box-and-whisker plots demonstrate the data distribution and group differences of the mean volume of left and right hemispheres in mm^3. In all plots, the middle line represents the median, and the upper and lower bounds of the boxes are the first and third quartiles, respectively. The whiskers extend from highest to lowest data point, and all data points are plotted. Representative atlas images are in sagittal, coronal and axial views (from left to right).

Table 3. Impact of maternal ART timing of initiation and maternal CD4 count during pregnancy on caudate and total grey matter volumes restricted to HIV-exposed and uninfected infants born to mothers on first-line triple ART

	Caudate		Total grey matter	
	Adjusted coefficient[a] (95% CI)	*p*-Value	Adjusted coefficient[a] (95% CI)	*p*-Value
ART initiation				
Before conception	*Reference*		*Reference*	
During pregnancy	−58.30 (−164.40 to 47.81)	0.263	−71.68 (−11,225 to 11,081)	0.989
CD4				
CD4 >500	*Reference*		*Reference*	
CD4 350–500	−25.52 (−150.70 to 99.67)	0.674	−5229 (−18,387 to 7930)	0.415
CD4 <350	−128.72 (−248.56 to −8.88)	0.037*	−15,023 (−27,619 to −2426)	0.022*

Note: Mean differences (adjusted regression coefficients with 95% CIs) and *p*-values for associations between brain volumes and maternal ART initiation timing in relation to pregnancy and CD4 cell count, restricted to those mothers on the same first-line ART regimen consisting of tenofovir + emtricitabine/lamivudine + efavirenz. ART timing was dichotomized into initiation before or during pregnancy. Total N = 25 (ART initiation before conception [n = 7], during pregnancy [n = 18]; CD4 >500 [n = 7], 350–500 [n = 6], <350 [n = 12]).
Abbreviations: ART, antiretroviral therapy; CI, confidence interval.
[a]Models were adjusted to include maternal CD4 cell count, ART initiation timing, child age at scan, child sex and intracranial volume (only ART initiation timing and CD4 are shown).
*$p < 0{\cdot}05$.
Post hoc the caudate was assessed bilaterally for the effect of CD4<350: left hemisphere: CD4 <350 adjusted coefficient: −131.41 (−260.99 to −1.84) p = 0.047; right hemisphere: CD4<350 adjusted coefficient: −126.03 (−258.30 to 6.24), p = 0.061.

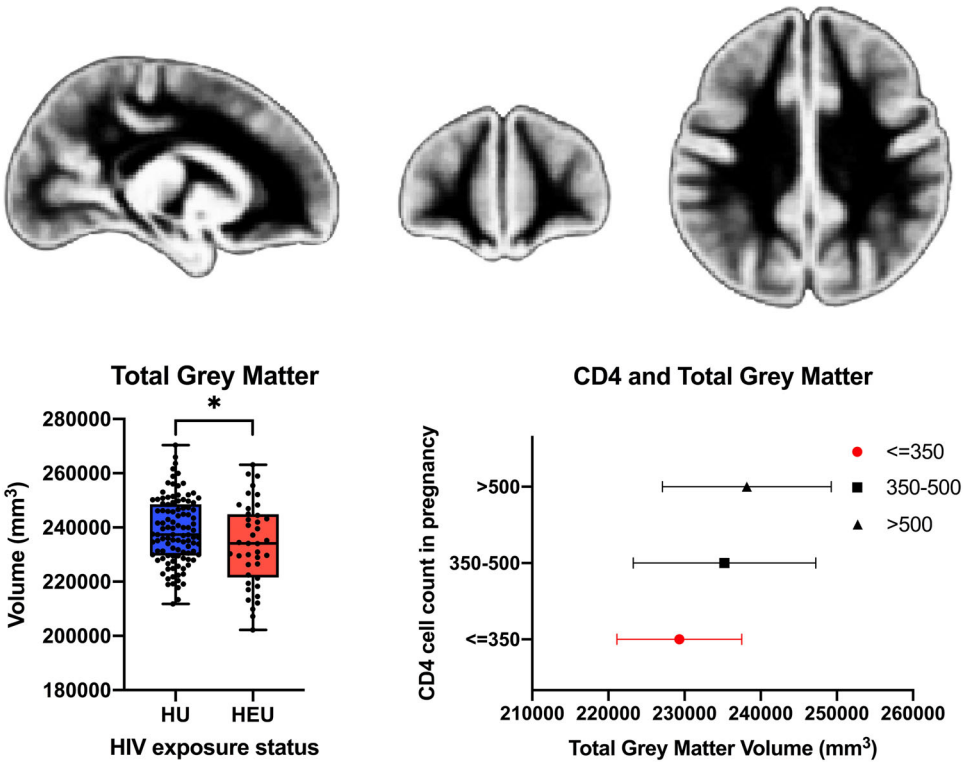

Figure 3. Assessment of the impact of HIV/ART exposure on total grey matter and associations with maternal immune status. (a) Comparison of total grey matter volume between infants who are HEU and HU. Total grey matter volumes are plotted comparing infants who are HEU (n = 40) and HU (n = 106) with an associated neonatal atlas of grey matter. *$p < 0.05$. We present box-and-whisker plots to demonstrate the data distribution. In all plots, the middle line represents the median, and the upper and lower bounds of the boxes are the first and third quartiles, respectively. The whiskers extend from highest to lowest data point, and all data points are plotted. (b) Association of maternal CD4 cell count with total grey matter volume in HEU infants. Mean volumes for children born to mothers with CD4 > 500 cells/mm^3 (n = 12); CD4 350–500 cells/mm^3 (n = 8); and CD4 < 350 cells/mm^3 (n = 13).

[47], suggesting that basal ganglia neurons may be particularly susceptible to the effects of HIV exposure [45]. The close proximity of the caudate to the cerebrospinal fluid [44] may also make it more vulnerable to changes to the *in utero* environment.

Our results also show that HIV exposure was associated with lower total grey matter volume, which builds on evidence of volume reductions reported in a small study of children aged 2 years [24]. However, this was a French retrospective study of children presenting with neurological symptoms, and the generalizability of these findings to the large HEU population in SSA is uncertain. Studies in children with HIV infection [30], adolescents [48] and adults [44] on ART have also reported smaller global grey matter volumes. Although mechanisms are likely to differ between children living with HIV and HEU, our data suggest that there may be similarities across neurobiological pathways for the impact of HIV on the developing brain in the context of exposure and infection; or the downstream effects of altered immune function in pregnancy may have a similar impact.

Various potential mechanisms are hypothesized by which maternal HIV exposure may impact a child's developing nervous system. In exploratory analyses, we found an association between maternal CD4 cell count during pregnancy and total grey matter and caudate volumes. Immune activation in pregnancy has been implicated in various neuropsychiatric disorders in the offspring [49], and associated with immunological dysregulation in children who are HEU [50] and altered brain development [51,52]. Importantly, the identified dose–response association between grey matter volume and maternal CD4 suggests that close maternal HIV management during pregnancy, including viral load monitoring and patient education to prevent immune compromise, may be key for promoting optimal fetal neural development.

Previous work has suggested potential neurotoxic effects of ART on the developing brain [24,53] and that timing of exposure may be a determinant of outcomes [40]. We investigated maternal ART regimen and timing of initial exposure in relation to conception. We found no relationship between ART exposure and brain volumes in this cohort. However, most mothers were on first-line triple therapy limiting statistical power to separate the effects of HIV and ART. In analyses restricted to HEU infants exposed to the same first-line ART, removing the potential diluting effect of different antiretrovirals, there was again no effect of ART initiation timing. Further work is needed in this area, particularly given the previously described associations between efavirenz and neurobehaviour [40,53] and the new WHO guidelines recommending dolutegravir-based ART [54]. While dolutegravir has improved efficacy [55], there were concerns raised in 2018

related to an increased risk of neural tube defects in women conceiving on dolutegravir [56,57]; however, recent data show that differences between dolutegravir- and non-dolutegravir regimens are no longer significant [10,58]. Long-term safety information on integrase strand transfer inhibitors is needed, and monitoring of child brain outcomes is critical.

Grey matter growth is fundamental for cognitive, language and motor skill development [15], and the caudate plays a role in motivation, movement, cognition and verbal fluency [59], as such, these structural deficiencies may have important neurodevelopmental implications. Postnatal maturation of the brain grey matter and subcortical structures occurs most rapidly in early infancy [15,17], and evidence from studies of premature infants has shown that deep grey brain volumes, particularly the caudate, predict academic outcomes in childhood [60,61]. Previous cohort studies have reported delayed neurocognitive development in children who are HEU compared to HU [12,62], although findings are inconsistent [14]. Delineating any differences in brain anatomy will help further understand the neurodevelopmental trajectories of children who are HEU. Our findings provide evidence for subtle neuroanatomical differences between infants who are HEU versus HU early in life. Future research is needed to further explore underlying causal pathways and the complex interactions between factors that impact brain maturation, including the role of biological, environmental and psychosocial variables [4].

Strengths of the study include a well-characterized cohort and an HEU group representative of other HEU populations across South Africa [63]. We present, to our knowledge, the largest neuroimaging study of children who are HEU during a period of importance for structural and functional development [17] that allows better differentiation between *in utero* versus postnatal risk factors than studies of older children. We also considered the potential impact of infant CMV infection. However, some important limitations of our study require consideration. Our sample size limited statistical power in the exploratory CD4 and ART analyses, and we were unable to examine maternal viral load. Although we excluded infants with known risk factors for neurodevelopment and adjusted for other covariates, we acknowledge the potential for residual confounding. More studies are needed to replicate these findings and assess generalizability. Finally, we only report a single imaging time-point and note the age of the cohort (2012–2015) as a limitation. Although overall PMTCT coverage at the time of this study was similar to many SSA countries currently [2], our cohort is pre-dolutegravir. The clinical relevance and longitudinal trajectories of the identified volumetric abnormalities need to be confirmed in future prospective studies, along with investigation of new ART regimens.

5 | CONCLUSIONS

We show that infants who are HEU had smaller caudate and total grey matter volumes compared to HU infants. Scans were conducted at a median of 21 days of life, limiting exposure to postnatal factors that may also influence brain development. The results are further strengthened by robustness after accounting for potential socio-economic confounders, and associations with maternal immune status. These findings suggest that antenatal HIV exposure may impact early brain structural development and maturation, and demonstrate that neuroanatomical changes are present soon after birth. This has important implications for understanding the risk of long-term neurodevelopmental impairment in the growing HEU population and suggests that improved HIV prevention, along with better maternal HIV management in pregnancy, may have the potential to optimize child brain development. Longitudinal follow up is needed to determine underlying mechanisms and whether these abnormalities persist into later childhood.

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COMPETING INTERESTS

The authors declare no competing interests.

AUTHORS' CONTRIBUTIONS

CJW processed the imaging data with supervision from NAG, and was responsible for statistical analysis, interpretation of results and drafting of the manuscript. KAD was responsible for the neuroimaging and with NAG, SY and DMG assisted with conception and manuscript revisions. AR assisted with data collection, AMR supported the data analysis, JPF and KLN provided imaging advice and all gave input into the manuscript. HJZ devised the Drakenstein Child Health study, DJS conceived the neuroimaging and maternal aspects, and both revised the manuscript critically for intellectual content. All authors have read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The de-identified data that support the findings of this study are available from the authors upon reasonable request as per DCHS cohort guidelines.

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SUPPORTING INFORMATION

Additional information may be found under the Supporting Information tab for this article:
Supporting Information
Table S1. Sociodemographic characteristics of children with neuroimaging versus those without in the Drakenstein Child Health Study
Table S2. Adjusted mean differences in grey matter volumes according to HIV exposure status excluding statistical outliers
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9

Chapter 9: Subcortical brain volumes and neurocognitive function of children with perinatal HIV exposure: a population-based cohort study in South Africa

(Research paper)

Subcortical brain volumes and neurocognitive function in children with perinatal HIV exposure: a population-based cohort study in South Africa

Summary

Chapter 9 presents the subcortical neuroimaging findings at age 2-3 years in the sixth research paper titled: 'Subcortical brain volumes and neurocognitive function in children with perinatal HIV exposure: a population-based cohort study in South Africa'. This follows the previous chapter and addresses objective 2 by examining the differences in subcortical brain volumes in HEU compared to HU children at the next age timepoint when extensive brain growth has occurred and child brains are over 80% of adult size. Subcortical volumes were correlated with neurocognitive function (objective 3) and with maternal CD4 cell count and viral load in pregnancy given the findings in infancy (secondary objective).

Overall, smaller subcortical grey matter volumes were found in HEU children compared to HU children aged 2-3 years. Analysis of individual structures demonstrated that HEU children had smaller volumes of basal ganglia nuclei, specifically the putamen, expanding on the results at 2-6 weeks. Additionally, HEU children had reduced hippocampus volume, a region known to be vulnerable to early-life exposures. Volumes in these regions were positively correlated with child language scores. These findings suggest that *in utero* HIV exposure may alter subcortical brain development with enduring impact, affecting clinical function. Furthermore, low maternal CD4 cell count and maternal viraemia (detectable HIV viral load) were associated with smaller volumes across regions, highlighting the importance of optimizing maternal ART to improve child brain outcomes.

My role in the conceptualisation, design, and neuroimaging data collection and image processing is documented in the Chapter 7 summary, and the neurodevelopment and HIV-related data collection is listed in Chapter 3. Furthermore, for this paper I conceived the manuscript; performed all statistical analyses with advice from A/Prof Andrea Rehman (statistician); undertook interpretation of results with advice from my supervisors; wrote the first manuscript draft, updated the paper in response to suggestions from co-authors; and co-ordinated the journal submission.

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Supplementary material

The supplementary material as detailed in the published article is available at https://doi.org/10.1093/ofid/ofae317 and listed in Appendix IX.

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Primary Supervisor	Professor Shunmay Yeung		

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MAJOR ARTICLE

Subcortical Brain Volumes and Neurocognitive Function in Children With Perinatal HIV Exposure: A Population-Based Cohort Study in South Africa

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Background. Children who are HIV-exposed and uninfected (HEU) are at risk for early neurodevelopmental impairment. Smaller basal ganglia nuclei have been reported in neonates who are HEU compared to HIV-unexposed (HU); however, neuroimaging studies outside infancy are scarce. We examined subcortical brain structures and associations with neurocognition in children who are HEU.

Methods. This neuroimaging study was nested within the Drakenstein Child Health Study birth cohort in South Africa. We compared (T1-weighted) magnetic resonance imaging–derived subcortical brain volumes between children who were HEU (n = 70) and HU (n = 92) at age 2–3 years using linear regression. Brain volumes were correlated with neurodevelopmental outcomes measured with the Bayley Scales of Infant and Toddler Development III.

Results. Compared to HU children, on average children who were HEU had 3% lower subcortical grey matter volumes. Analyses of individual structures found smaller volume of the putamen nucleus in the basal ganglia (−5% difference, $P = .016$) and the hippocampus (−3% difference, $P = .044$), which held on adjustment for potential confounders ($P < .05$). Maternal viremia and lower CD4 count in pregnancy were associated with smaller child putamen volumes. Children who were HEU had lower language scores than HU; putamen and hippocampus volumes were positively correlated with language outcomes.

Conclusions. Overall, children who are HEU had a pattern of smaller subcortical volumes in the basal ganglia and hippocampal regions compared to HU children, which correlated with language function. Findings suggest that optimizing maternal perinatal HIV care is important for child brain development. Further studies are needed to investigate underlying mechanisms and long-term outcomes.

Keywords. basal ganglia; child brain structure; HIV exposure; immune function; language.

The population of children born to mothers living with human immunodeficiency virus (HIV) who remain HIV-free is expanding, estimated at 16 million worldwide [1]. In some sub-Saharan African countries, >1 in 5 children are born HIV-exposed and uninfected (HEU) [1]. While infant HIV infection is known to affect child development, perinatal exposure to HIV without infection is also a risk factor for adverse outcomes. Children who are HEU have been found to have subtle impairment in early neurodevelopment when compared to their HIV-unexposed (HU) peers [2, 3]. In particular, poorer language outcomes have been noted by 24 months in large studies [4, 5], and results from a recent meta-analysis found that children who are HEU have lower expressive language and gross motor scores compared to HU children across the early years [3].

Understanding the underlying neurobiology may inform prevention and intervention strategies. However, studies examining brain structure of children who are HEU are scarce, and systematic reviews highlight the lack of neuroimaging research [3, 6]. Recently, infants who are HEU were reported to have smaller basal ganglia nuclei compared to HU infants at 2–6 weeks of age in the Drakenstein Child Health Study (DCHS)

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[7] and another birth cohort study in South Africa [8]; reduced brain volumes were associated with maternal immunocompromise, as measured by lower CD4 cell counts in pregnancy [7], HIV disease severity, and antiretroviral therapy (ART) timing [8]. The basal ganglia nuclei, notably the caudate and putamen, are integral to networks serving cognition and behavior [9, 10], and this subcortical region is known to be vulnerable in people with HIV infection [11]. Separately, a few studies of older children who are HEU have found altered white matter using diffusion tensor imaging as well as differences in neurometabolites on magnetic resonance spectroscopy [12, 13], suggesting that brain development may continue to be affected. However, to our knowledge, there are no studies quantitatively reporting on perinatal HIV exposure and subcortical brain volumes beyond infancy.

The first 3 years of life, from conception through early childhood, are fundamental for brain development, when >80% of subcortical brain growth happens [14]. The age-related changes in brain structure that occur during this period are complex, establishing the foundations of cognitive and language development [15]. In the context of HIV, brain development may be affected by a multitude of factors including HIV and ART exposure, biological factors, an altered uterine environment, and socioenvironmental variables. Understanding the association between early brain structure and function in the context of HIV exposure may help to elucidate underlying mechanisms behind neurodevelopmental impairment. In this study of the DCHS population-based birth cohort, we aimed to investigate whether associations of HIV exposure with subcortical brain structures are evident at age 2–3 years, and whether volumetric differences are related to maternal HIV disease severity and neurodevelopmental outcomes. We hypothesized that children who are HEU would show smaller brain volumes in the basal ganglia region, as reported in infants who are HEU [7], and that these would correlate with neurocognitive function.

MATERIALS AND METHODS

Study Design and Participants

The DCHS is a South African birth cohort study established to investigate the determinants of child health [16, 17]. The population has an antenatal HIV prevalence of 21%. Between 2012 and 2015, pregnant women aged 18 years or older attending routine antenatal appointments were recruited from 2 public sector primary healthcare clinics with written informed consent. Mother–child pairs are followed up prospectively.

This nested longitudinal neuroimaging substudy enrolled children from the DCHS [18, 19]. Between 2012 and 2015, a sample of infants aged 2–6 weeks had magnetic resonance imaging (MRI) scans. From 2016 to 2018, these children were invited to return for a further MRI. Additional children aged 2–3 years were invited based on risk factor exposure. The subgroup had specific exclusion criteria of conditions known to impact brain development including prematurity, birth factors (Apgar score <7 at 5 minutes or neonatal intensive care admission), medical comorbidities, maternal use of illicit drugs during pregnancy, child HIV infection, or MRI contraindications. Additional written informed consent was obtained for the neuroimaging.

Procedures

HIV Care. HIV testing of all mothers was performed routinely during pregnancy and the postnatal period, and children were tested at 6 weeks, 9 months, and 18 months and after cessation of breastfeeding [20]. Mothers living with HIV were initiated on ART per local guidelines at the time. Prior to May 2013, mothers were given triple ART or zidovudine monotherapy from 14 weeks' gestation dependent on WHO clinical stage and CD4 cell count. From 2013, all pregnant women were initiated on triple ART for life. Children with HIV exposure received prophylaxis using nevirapine alone or in combination with zidovudine. We extracted data on maternal CD4 cell count and viral load during pregnancy from folders and the online National Health Laboratory Service system.

Sociodemographic Data. Sociodemographic data, birth anthropometry, gestational age, and infant feeding data were collected using standard protocols [21]. Weight, height, and head circumference were measured during the scan visit. Maternal alcohol use during pregnancy was recorded as a composite measure derived from the Alcohol, Smoking and Substance Involvement Screening Test in pregnancy and 2 retrospective self-report questionnaires [17, 18].

Neuroimaging

MRI Data Acquisition and Processing. Structural T1-weighted images were acquired on a 3-Tesla Siemens Skyra scanner at the Cape Universities Body Imaging Centre. Given the need to limit motion for high-quality scans, the imaging was carried out during natural sleep without sedation or general anesthetic. Scans were arranged for the lunchtime nap, or later in the day when the children were more likely to sleep [19]. A 3D MEMPRAGE (multi-echo magnetization prepared rapid acquisition gradient echo) scan in sagittal orientation was used: repetition time 2530 ms, echo time 1.69, 3.54, 5.39, 7.24 ms, inversion time 1100 ms, flip angle 7.0°, voxel size 1.0 mm^3, 176 slices, field of view: 224 × 224 × 176 mm.

Imaging data processing involved a series of automated steps using FreeSurfer software version 6.0 (http://surfer.nmr.mgh.harvard.edu/) [22] at the Centre for High Performance Computing (Cape Town). Subcortical volume and intracranial volume (ICV) were extracted from segmented images. Parcellation into the 7 subcortical regions (caudate, putamen, pallidum, thalamus, hippocampus, amygdala, nucleus accumbens) was performed [23].

Quality Control. Quality control was performed blinded to maternal HIV status. MRI scans were reported by a radiologist; incidental findings were reviewed by a pediatric neurology consultant and appropriate care was arranged using local clinical pathways. Images were visually inspected for artefacts at the outset and segmentation errors were assessed following processing. The ENIGMA protocol for subcortical brain regions (http://enigma.ini.usc.edu/protocols/imaging-protocols/) was used to assess for outliers.

Neurodevelopmental Assessments

Neurodevelopment was measured using the Bayley Scales of Infant and Toddler Development, third edition (BSID-III) tool, which has been validated in South Africa [24, 25]. Trained assessors blinded to HIV exposure status assessed cognitive, language, and motor outcomes of children by direct observation. Quality control and monitoring were implemented. Age-adjusted composite scores for cognitive, language, and motor domains were used. While neurodevelopment was assessed across the whole DCHS cohort, we report the results for the imaging subsample here.

Statistical Analysis

We conducted targeted analyses to address our hypotheses. Robust simple and multiple linear regression models were constructed with HIV exposure status as the independent factor and (*i*) total subcortical volume (summing all 7 regions together) and (*ii*) each individual region volume as the dependent factors. Three variables were identified a priori as covariates (age at scan, child sex, and ICV), given their known association with brain structure and to account for overall head (brain) size differences. Given the known influence of other socioenvironmental factors on neurodevelopment, additional covariates (maternal education, maternal age at birth, and household income) were identified using a directed acyclic graph constructed previously [5]. To restrict the number of statistical comparisons when analyzing individual subcortical regions, we used the mean of left and right hemispheres and examined each hemisphere separately where significant associations were identified. Significance level was set to $P < .05$. We calculated Cohen d effect sizes and the percentage difference using the mean absolute difference in volume between children who were HEU and HU relative to the mean HU volume.

To further improve our understanding of the relationship between HIV exposure and neurodevelopment, we next investigated the relationship between subcortical brain volumes and cognitive, language, and motor outcomes using Pearson correlations. We report correlation coefficients and 95% confidence intervals (CIs). Additionally, we explored the associations of maternal CD4 cell count and viral load in pregnancy with subcortical regional volumes that had significant differences by HIV exposure status. Sensitivity analyses were performed (*i*) to investigate how breastfeeding might have influenced the main effect of HIV exposure status on volumetric measures and (*ii*) to check the results held on excluding imaging outliers. All statistical analyses were performed using Stata software version 14.2 (StataCorp, College Station, Texas).

Data Availability

The de-identified data that support the findings of this study are available from the authors upon reasonable request as per DCHS cohort guidelines.

RESULTS

Demographics

A total of 162 children with high-resolution scans were included, 70 HEU and 92 HU (Figure 1). The mean age at scan was 34.1 months (standard deviation, 1.7 months) and 94 (58%) were male. Sociodemographic, clinical, and neurodevelopmental data are reported in Table 1. There were no group differences in maternal socioeconomic parameters and birth outcomes, although mothers with HIV were older (29.6 vs 26.8 years). Mothers with HIV were less likely to breastfeed (40% vs 90%), although mean duration of exclusive breastfeeding was similar (1.6 vs 2.0 months) in those who did commence breastfeeding. Overall, 69 of 70 (99%) children HEU were born to mothers taking triple ART, with the most common regimen being tenofovir, emtricitabine, and efavirenz. More than half of mothers initiated ART in pregnancy, with 44% initiating ART prior to pregnancy. During pregnancy, median maternal CD4 cell count was 476 cells/μL; 23% of mothers had detectable viral loads. Most infants with HIV exposure had nevirapine prophylaxis (80%), while the remaining had nevirapine and zidovudine (Table 2) according to local standard of care.

On examination of neurodevelopmental outcomes, children who were HEU had poorer language outcomes, scoring approximately 5 points lower than HU children (81.8 vs 86.3, $P = .03$). Cognitive (85.0 vs 87.4, $P = .12$) and motor scores (93.2 vs 94.5, $P = .51$) were similar. ICV did not significantly differ across groups (Table 1).

Subcortical Volumes and HIV Exposure Status

The comparisons of subcortical volumes between children who were HEU versus HU are shown in Table 3 and illustrated in Figure 2. On average, we found that children who are HEU had significantly smaller total subcortical grey matter volume compared to HU children (46 929 vs 48 311 mm^3, $P = .049$; Cohen d effect size, −0.31 [95% CI, −.63 to −.00]), which represented a difference of 2.9%. The findings held after adjusting for multiple covariates including child age, sex, ICV, maternal education, household income, and maternal age (adjusted mean difference, −945.92 mm^3 [95% CI, −1774.06 to −117.78 mm^3], $P = .025$).

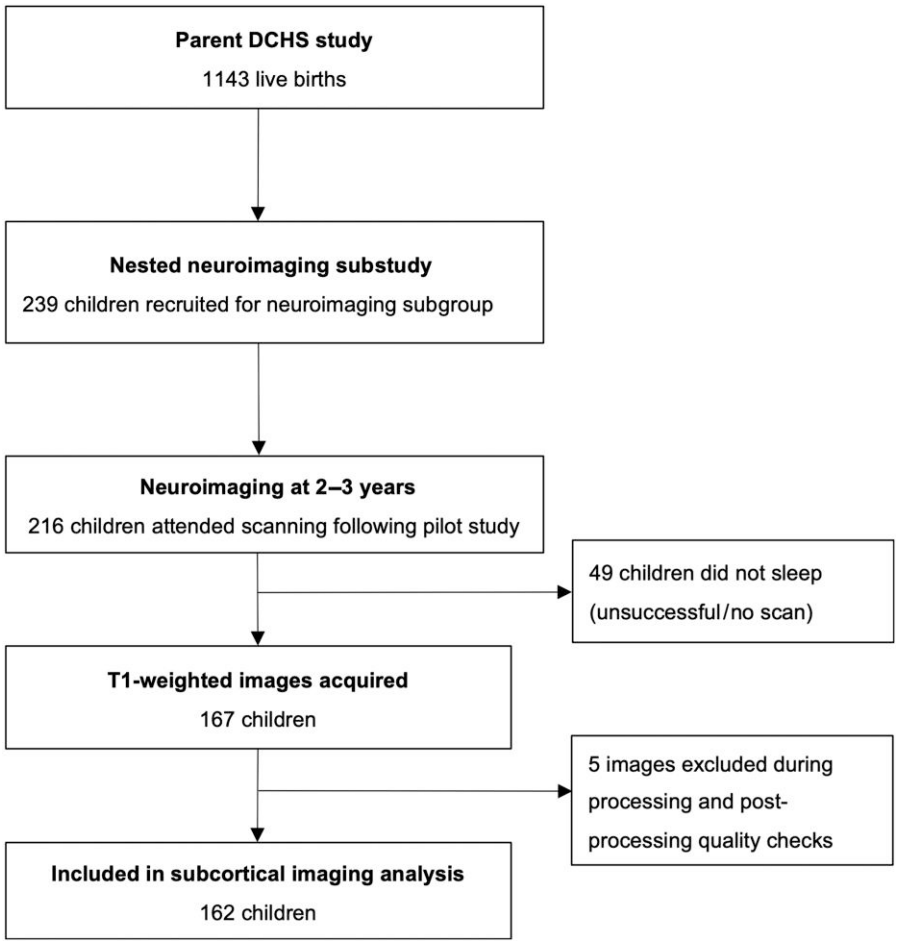

Figure 1. Drakenstein Child Health Study (DCHS) cohort flowchart of children included in this analysis.

On examination of individual structures, children who were HEU had lower putamen volume compared to HU children (−4.7%; 4381 vs 4597 mm^3, $P = .016$; effect size, −0.37 [−.69 to −.06]). Findings held on adjusting for potential confounders (adjusted mean difference, −181.19 mm^3 [95% CI −342.46 to −19.92 mm^3], $P = .028$) and results were similar when analyzed separately by hemisphere. Compared to HU, children who were HEU also had lower hippocampal volume (−3.4%; 3043 vs 3149 mm^3, $P = .044$; effect size, −0.31 [95% CI, −.62 to .00]), a finding that remained on adjustment (adjusted mean difference, −86.34 mm^3 [95% CI, −171.80 to −0.87 mm^3], $P = .048$). On analyzing the separate hemispheres (Supplementary Table 1), similar adjusted differences were seen (left hippocampus: −88.11 mm^3 [95% CI, −169.80 to −6.42 mm^3], $P = .035$; right hippocampus: −84.56 mm^3 [95% CI, −183.30 to 14.18 mm^3], $P = .093$); however the right hippocampus lost statistical significance. There were no significant differences in other subcortical volumes (thalamus, pallidum, amygdala, caudate, nucleus accumbens; all $P > .05$).

Subcortical Volumes and Neurodevelopmental Outcomes

Next, we explored the correlation between brain structure and cognitive, language, and motor outcomes. The results from the correlation analyses are presented in Table 4. Putamen and hippocampal volumes were found to be positively correlated with language, with modest effect sizes (putamen: $r = 0.20$ [95% CI, .04 to .36], $P = .017$; hippocampus: $r = 0.21$ [95% CI, .05 to .37], $P = .012$). There were no structural correlations with development in other domains.

Association With Maternal HIV Disease Severity (CD4 Cell Count and Viral Load in Pregnancy)

In exploratory analyses examining associations between subcortical volumes and maternal CD4 and viral load during pregnancy, we found that lower maternal CD4 (<350 cells/μL) was significantly associated with smaller putamen volumes (Figure 3; Supplementary Table 2). Similarly, maternal viremia (detectable HIV viral load) was associated with smaller total subcortical and putamen volumes with a dose-response pattern across all regions (Figure 3; Supplementary Table 2).

Table 1. Sociodemographic and Clinical Characteristics of Children According to HIV Exposure

Variable	HEU Children (n = 70)	HU Children (n = 92)	*P* Value
Maternal sociodemographic characteristics			
Household income per month (>1000 rand [>~75 USD])	46 (65.71%)	65 (70.65%)	.50
Maternal education (completed secondary)	19 (27.14%)	35 (38.04%)	.15
Employment status (employed)	17 (24.29%)	27 (29.35%)	.47
Maternal alcohol use during pregnancy	7 (13.46%)	17 (21.25%)	.26
Maternal age at birth, y, mean (SD)	29.57 (4.94)	26.80 (5.81)	.002*
Maternal clinical characteristics			
Maternal hospitalization during pregnancy	3 (4.29%)	2 (2.17%)	.65
Maternal history of TB	7 (10.14%)	4 (4.35%)	.21
Child sociodemographic characteristics			
Child age at scan, mo, mean (SD)	33.83 (1.79)	34.28 (1.65)	.10
Child sex (male)	46 (65.71%)	48 (52.17%)	.08
Gestational age at birth, wk, mean (SD)	38.43 (2.46)	39.17 (2.51)	.06
Any breastfeeding	28 (40.00%)	83 (90.22%)	<.001*
Exclusive breastfeeding	25 (35.71%)	55 (59.78%)	.002*
Exclusive breastfeeding duration, wk, mean (SD)	1.56 (2.09)	2.03 (1.51)	.10
Child neurodevelopmental outcomes			
Language development, mean (SD)	81.82 (10.68)	86.25 (11.84)	.03*
Cognitive development, mean (SD)	85.00 (9.30)	87.44 (9.29)	.12
Motor development, mean (SD)	93.22 (11.38)	94.47 (10.55)	.51
Anthropometry			
Birth head circumference, cm, mean (SD)	33.31 (1.92)	33.90 (1.97)	.06
Birthweight, kg, mean (SD)	3.04 (0.60)	3.14 (0.55)	.29
Low birth weight	10 (14.29%)	9 (9.78%)	.38
Weight at scan, kg, mean (SD)	13.94 (2.02)	13.79 (1.90)	.63
Height at scan, cm, mean (SD)	90.63 (4.04)	91.80 (3.91)	.08
Head circumference at scan, cm, mean (SD)	49.78 (1.76)	49.75 (1.79)	.97
Neuroanatomical variables			
Total intracranial volume, cm^3, mean (SD)	1208.46 (116.49)	1218.68 (119.14)	.59

Data are presented as No. (%) unless otherwise indicated. Continuous variables were compared with unpaired *t* tests; categorical variables were compared with χ^2 or Fisher exact test if n < 5. Percentages are cited among those with nonmissing values. Missing data: relationship status (n = 1), alcohol (n = 30), maternal history of TB (n = 1), head circumference at birth (n = 2), head circumference at scan (n = 1), height at scan (n = 21), cognitive composite score (n = 16), language composite score (n = 24), motor composite score (n = 21).

Abbreviations: HEU, HIV-exposed and uninfected; HU, HIV-unexposed; HIV, human immunodeficiency virus; SD, standard deviation; TB, tuberculosis; USD, United States dollars; VL, viral load.

*$P < .05$.

Sensitivity Analyses

Associations between HIV exposure and smaller putamen and hippocampus volumes remained after adjusting for any breastfeeding and duration of exclusive breastfeeding (Supplementary Table 3*A* and 3*B*). Similar results were also found in sensitivity analyses excluding imaging outliers (Supplementary Table 4).

DISCUSSION

This study describes the association of perinatal HIV exposure and subcortical brain volumes through early childhood. On average, children who were HEU had smaller subcortical grey matter volumes compared to HU children at 2–3 years, notably in the basal ganglia, building on findings in infancy. Additionally, children who were HEU had lower hippocampal volumes at 2–3 years, a region known to be vulnerable to early-life exposures. These regional subcortical volumes showed small but significant correlations with language outcomes. Overall, the results indicate that HIV exposure in pregnancy may affect early subcortical brain development, with enduring impact and implications for language. Finally, identified associations between maternal CD4 and viral load in pregnancy with subcortical volumes suggest a role for maternal immunosuppression and HIV disease severity in child brain maturation.

The basal ganglia are a collection of nuclei in the deep grey matter, the largest components being the caudate and putamen, which have roles in modulating motor, cognition, behavior, and language processing [9, 10]. At 2–3 years, we found reduced putamen volume in children who are HEU compared to HU. This expands on our prior findings showing smaller caudate volume, the adjoining nucleus, in neonates who are HEU compared to HU [7], as well as the results of another South African study reporting smaller putamen volume in

Table 2. Maternal and Child HIV Variables

Variable	HEU Children (n = 70)
Maternal HIV diagnosis	
Prior to pregnancy	51 (72.86%)
During pregnancy	19 (27.14%)
Maternal CD4 cell count during pregnancy[a]	
Median (IQR) cell count, cells/μL	476 (344–677)
<350 cells/μL	17 (27.87%)
350–500 cells/μL	16 (26.23%)
≥500 cells/μL	28 (45.90%)
Maternal viral load in pregnancy[a]	
<40 copies/mL	44 (77.19%)
≥40 copies/mL	13 (22.81%)
Maternal ART	
Monotherapy with zidovudine	1 (1.43%)
Triple therapy	69 (98.57%)
Maternal ART initiation	
Prior to pregnancy	31 (44.29%)
During pregnancy	39 (55.71%)
Infant prophylaxis	
Nevirapine alone	56 (80.00%)
Nevirapine + zidovudine	14 (20.00%)

Data are presented as No. (%) unless otherwise indicated. Percentages are cited among those with nonmissing values. Missing data: CD4 count (n = 9), viral load (n = 13).

Abbreviations: ART, antiretroviral therapy; HEU, HIV-exposed and uninfected; HIV, human immunodeficiency virus; IQR, interquartile range.

[a]Where multiple results were available, the lowest maternal CD4 count within 1 year prior to birth and 3 months postbirth was used to reflect maternal immunosuppression in pregnancy and maximize sample numbers. Maternal viral load during pregnancy was classified into <40 (undetectable) and ≥40 (detectable) copies/mL; where there was >1 measurement, we selected the highest as a reflection of the most severe disease exposure.

infants who are HEU compared to HU and reduced caudate volume in HEU newborns born to mothers initiating ART in pregnancy [8]. Together the putamen and caudate represent the dorsal striatum of the basal ganglia [26], which is closely linked to the cortex through cortico-striatal pathways [27]. Our results suggest that changes in this region seen in infancy may persist and develop with brain maturation. These findings are consistent with reports of lower basal ganglia metabolites in school-aged children who are HEU [28], qualitative basal ganglia abnormalities in 2-year-old HEU children presenting with neurological symptoms [29], and basal ganglia involvement commonly reported in children with HIV [11].

Individual subcortical structures have varying growth trajectories, and our findings may indicate differential regional vulnerability during windows of brain maturation. The putamen displays earlier maturation relative to other regions, showing the greatest volume change from 3 to 12 months with an approximately 2-fold increase over that period [30]. Increased metabolic activity during periods of maximal growth may make structures more vulnerable to early insults, or the variation associated with these rapid changes may mean that differences are detectable earlier than in other regions. At 2–3 years, we also found lower hippocampus volume in children who are HEU compared to HU. The hippocampus is unique in displaying both neurogenesis and neuroplasticity throughout the postnatal period into adult life [31]. As a result, it is more sensitive to brain insults and stressors than other structures, and smaller left hippocampal volumes have also been seen in children living

Table 3. Mean Differences in Subcortical Brain Volumes According to HIV Exposure

Region	Mean (SD) Volume, mm^3 HEU (n = 70)	HU (n = 92)	Unadjusted Difference (95% CI)	*P* Value	Effect Size Cohen d (95% CI)	Fully Adjusted[a] Difference (95% CI)	*P* Value	Effect Size Cohen d (95% CI)
Total subcortical volume	46 929 (4597)	48 311 (4092)	−1381.89 (−2755.27 to −8.52)	.049*	−0.31 (−63 to −.00)	−945.92 (−1774.06 to −117.78)	.025*	−0.22 (−.53 to .10)
Thalamus	5788 (602)	5919 (548)	−130.51 (−311.86 to 50.85)	.157	−0.23 (−.54 to .09)	−90.87 (−209.98 to 28.24)	.134	−0.16 (−.47 to .15)
Caudate	3246 (525)	3361 (472)	−114.66 (−272.16 to 42.83)	.152	−0.23 (−.54 to .08)	−44.60 (−172.51 to 83.32)	.492	−0.09 (−.40 to .22)
Putamen	4381 (576)	4597 (543)	−215.98 (−391.98 to −39.97)	.016*	−0.37 (−.69 to −.06)	−181.19 (−342.46 to −19.92)	.028*	−0.32 (−.63 to −.00)
Pallidum	1535 (225)	1574 (200)	−39.26 (−106.35 to 27.84)	.250	−0.19 (−.50 to .13)	−19.16 (−77.36 to 39.03)	.516	−0.09 (−.40 to .22)
Hippocampus	3043 (321)	3149 (341)	−106.10 (−209.42 to −2.78)	.044*	−0.31 (−.62 to .00)	−86.34 (−171.80 to −.87)	.048*	−0.26 (−.57 to .06)
Amygdala	1242 (159)	1281 (160)	−38.80 (−88.70 to 11.10)	.127	−0.24 (−.55 to .07)	−31.20 (−77.13 to 14.73)	.182	−0.19 (−.51 to .12)
Nucleus accumbens	587 (90)	572 (83)	14.92 (−12.47 to 42.30)	.284	0.17 (−.14 to .48)	17.15 (−8.63 to 42.93)	.191	0.20 (−.11 to .51)

Regional volumes (mean of left and right hemispheres), mean differences (regression coefficients unadjusted and fully adjusted in multiple regression models), *P* values, and effect sizes for associations between brain volumes and HIV exposure. Residuals were assessed for each model using quantile-quantile plots and were normally distributed.

Abbreviations: CI, confidence interval; HEU, HIV-exposed and uninfected; HU, HIV-unexposed; HIV, human immunodeficiency virus; SD, standard deviation.

[a]Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income, and maternal age.

*$P < .05$.

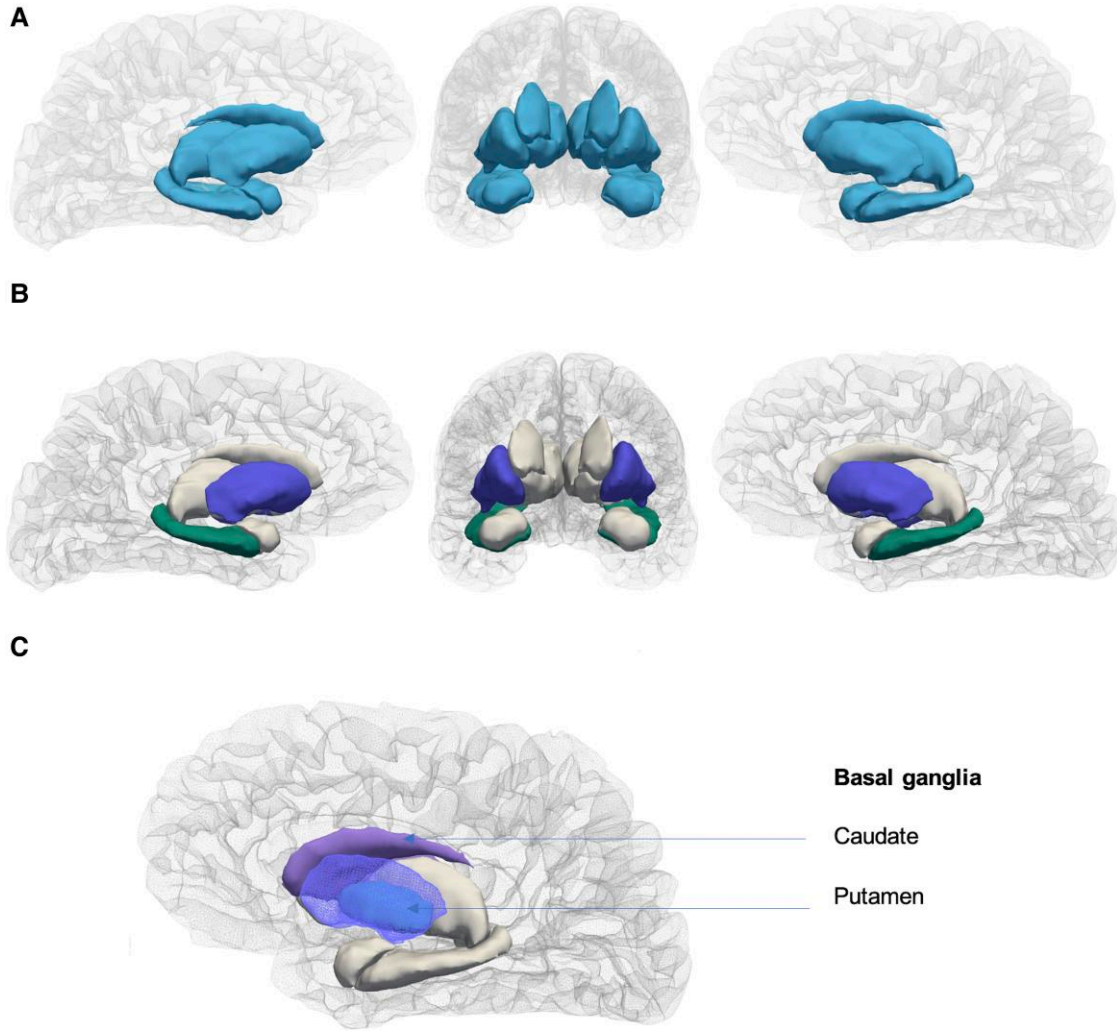

Figure 2. Representation of subcortical structures highlighting those affected in children with perinatal HIV exposure. *A*, All subcortical structures. *B*, Individual structures with significant differences between children who are HIV-exposed and uninfected and HIV-unexposed at 2–3 y. Dark blue: putamen; dark green: hippocampus. *C*, Detailed view of the basal ganglia.

with HIV [32]. These characteristics underlie the role of the hippocampus in learning and memory formation as well as verbal fluency, and therefore any early deviations from typical maturational trajectories may have implications for later neurocognitive function.

In support of this interpretation, we found an association between subcortical putamen and hippocampus volumes with language development in the present investigation. Neurocognitive functioning is increasingly understood to be dependent on distributed neural networks, which include the basal ganglia and hippocampus [33]. Consistent with this, prior studies have found a relationship between putamen volume, language, and verbal learning [9, 34] and associations between basal ganglia nuclei growth with IQ and language scores [10, 35], potentially indicating early growth of these structures as markers of long-term outcomes. A recent meta-analysis also identified basal ganglia anomalies in developmental language disorders [36]. However, caution needs to be taken when interpreting the clinical implications of brain volume differences, and we note the correlations are small. Altered basal ganglia structure is associated with disrupted cortico-striatal circuitry, which is critical to higher-order cognitive functions, language usage, and learning [37]. Thus, the associations may be due not only to pathology directly related to subcortical volumes, but also to any part of the several parallel loops involving these structures, potentially explaining the smaller effect sizes for the individual subcortical structures.

Early brain development involves myelination, synaptogenesis, pruning, and synaptic modification, which are affected by genetic, biological, environmental, and infectious factors [38]. These processes of brain maturation may be particularly sensitive to direct effects of HIV exposure as well as to the associated effects of

Table 4. Correlations Between Subcortical Brain Volumes and Developmental Domains

	Bayley Scales of Infant and Toddler Development								
	Language Development			Cognitive Development			Motor Development		
Region	*r*	(95% CI)	*P* Value	*r*	(95% CI)	*P* Value	*r*	(95% CI)	*P* Value
Thalamus	0.13	(−.04 to .29)	.121	−0.00	(−.16 to .16)	.999	0.14	(−.03 to .30)	.100
Caudate	0.15	(−.01 to .31)	.073	−0.03	(−.19 to .13)	.708	0.14	(−.03 to .30)	.101
Putamen	0.20	(.04 to .36)	.017*	0.16	(−.00 to .32)	.052	0.13	(−.04 to .28)	.140
Pallidum	0.14	(−.03 to .30)	.111	0.01	(−.15 to .17)	.899	0.06	(−.11 to .22)	.474
Hippocampus	0.21	(.05 to .37)	.012*	0.09	(−.07 to .25)	.283	0.16	(−.00 to .32)	.055
Amygdala	0.09	(−.08 to .25)	.301	0.04	(−.13 to .20)	.647	0.07	(−.09 to .24)	.383
Nucleus accumbens	−0.02	(−.18 to .15)	.860	−0.04	(−.20 to .12)	.641	0.02	(−.15 to .18)	.850

Shown are Pearson correlations and 95% CIs. n = 138 for language development; n = 146 for cognitive development; n = 141 for motor development.

Abbreviation: CI, confidence interval.

*$P < .05$.

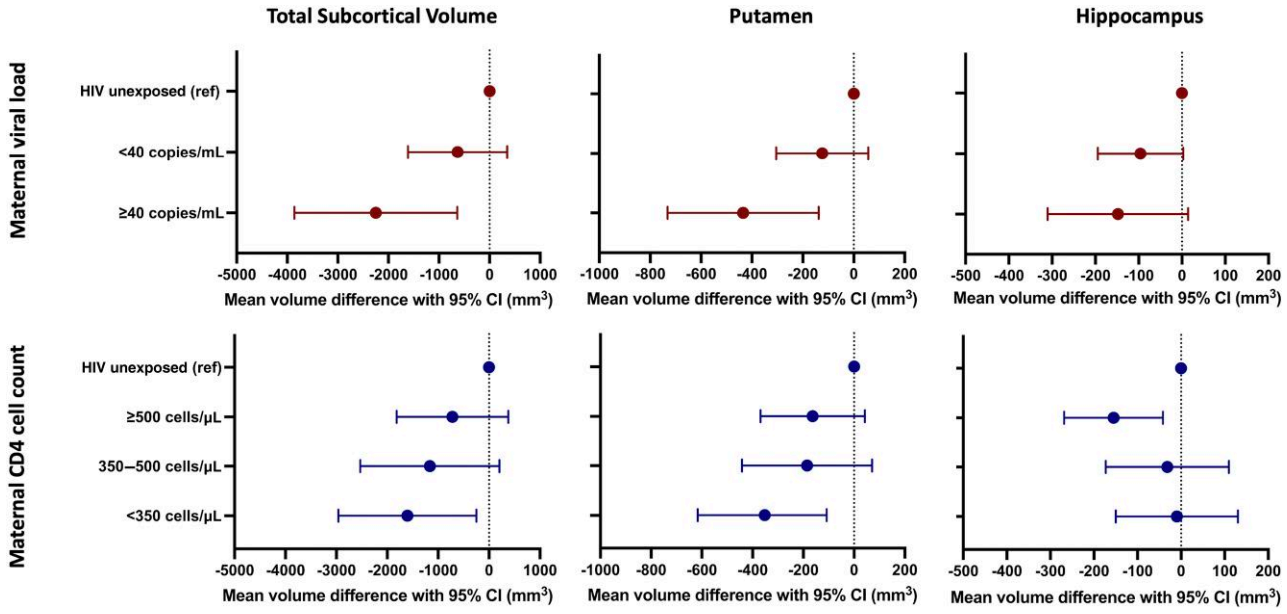

Figure 3. Associations of maternal CD4 cell count and viral load in pregnancy with child total subcortical, putamen, and hippocampus volumes. Mean volume differences are plotted with 95% confidence intervals (CIs) in mm^3 compared to the HIV–unexposed reference group. Mean volume differences shown are fully adjusted for covariates as detailed in Supplementary Table 2.

the virus on immune activation and inflammatory cytokines [39, 40]. We show an association between lower maternal CD4 count during pregnancy and smaller putamen volume in HEU children, similar to the relationship seen in early infancy [7]. Additionally, we found that maternal HIV viremia was associated with smaller subcortical and putamen volumes. This suggests the basal ganglia may be affected by HIV disease, either directly or via corresponding immune changes. HIV-associated structural deficits, including reduced putamen volumes in infants, have also been shown to be associated with maternal HIV viral load in pregnancy in neonates who are HEU [8], as well as to persist after ART initiation in children with HIV infection [32], suggesting a role for immune activation and/or inflammation in both HIV infection and exposure. Previously we reported a neuroinflammatory pattern on magnetic resonance spectroscopy in HEU children at 2–3 years [12], fitting with this hypothesis.

HIV exposure frequently occurs within the context of a range of risk factors, HIV-related and universal, many of which may affect the developing brain [39]. In particular, the role of ART is important to consider. Most mothers were taking ART regimens including efavirenz. Given that efavirenz has been linked to central nervous system adverse effects in people with HIV [41] and to worse HEU child language outcomes [42], neurotoxicity could be a contributing factor. Dolutegravir is now

the recommended first-line regimen and new studies are required to assess integrase inhibitors, considering whether the improved efficacy at reducing viral load may translate to immune protection and better outcomes. We did not find that breastfeeding drove the differences in this analysis, which aligns with data from other fully breastfed cohorts [43]. However, we recognize that the neuroimaging findings need to be replicated in contexts with higher breastfeeding rates. Overall, there are likely multifactorial etiologies, and studies are needed to understand mechanisms and tease apart the effects of HIV, ART, and other socioenvironmental risk.

The findings suggest a few strategies for optimizing outcomes. While the focus needs to remain on prevention of HIV acquisition in mothers and children, the maternal CD4 and viral load results suggest that improved perinatal HIV management to reduce severe disease and immunological compromise may positively impact child neural development. This could include opportunities such as preparation for pregnancy, viral load monitoring, parent education, and ART adherence. The results highlight incentives for early booking in pregnancy to ensure prompt diagnosis and ART initiation, preventing late-presenting HIV disease [44], and the potential role of new antiretrovirals in improving outcomes. Finally, the findings represent a comparison of average group differences, and not all children who are HEU will have developmental difficulties. Therefore, implementing developmental assessments to monitor at-risk children to detect any developmental delay early and refer for interventions will help to ensure optimal health outcomes.

Strengths of this study include the comprehensive neuroimaging and neurodevelopmental assessments; the well-characterized sample with demographically appropriate controls; and the window of measurement in the first 3 years, a critical period of brain growth. However, this study has limitations, some of which are inherent to neuroimaging studies. The relatively small sample size, although large for an MRI study, may have reduced precision and power to detect an effect. Findings need to be replicated, particularly the exploratory analyses, and incorporating a range of immune factors in further research will highlight the full role of immune function. Second, there were some demographic differences between groups in maternal age and breastfeeding. We adjusted for covariates and conducted sensitivity analyses of breastfeeding, which suggested minimal confounding. However, we acknowledge the potential for residual bias and impact of other unmeasured factors, including cytomegalovirus infection and anemia in pregnancy, that need further investigation. Third, ART was not formally explored and the underlying mechanisms need investigation. Finally, we performed cross-sectional analyses, as only 33% of children had scans in infancy and 2–3 years; research is ongoing at older ages so that we will be able to map brain structure longitudinally. Overall, the clinical relevance and trajectories of the identified volumetric differences need to be confirmed in future prospective studies.

In conclusion, at age 2–3 years, children who are HEU showed small differences in subcortical volumes compared to HU children, particularly in the basal ganglia region, similar to those seen in infancy. Our findings represent the first prospective cohort study to report subcortical volumes of children who are HEU at an age when 80% of subcortical growth has occurred [14]. The results suggest possible biological pathways by which HIV exposure might contribute to impaired language development and establish the basal ganglia and hippocampus as regions of vulnerability. Given that the largest part of brain development occurs within the first 3 years of life, influencing future academic and health outcomes, further work will be important to understand contributing mechanisms and how to optimize brain development of this vulnerable population.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Ethical approval. This study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009; 525/2012; 044/2017), the Western Cape Department of Provincial Health Research Committee, and the London School of Hygiene and Tropical Medicine Observational/Interventions Research Ethics committee (11903). Written informed consent was obtained at DCHS recruitment, and mothers are reconsented annually. Additional written informed consent was obtained from the parent/guardian at the neuroimaging visit.

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Chapter 10: Association of *in utero* HIV exposure with child brain structure and language development: a South African birth cohort study

(Research paper)

Association of *in utero* HIV exposure with child brain structure and language development: a South African birth cohort study

Summary

Chapter 10 presents the final research paper titled: 'Association of *in utero* HIV exposure with child brain structure and language development: a South African birth cohort study.' This paper examines the cortical brain structure (cortical thickness and surface area) of HEU compared to HU children, and the association with language development at 2-3 years of age, addressing objectives 2 and 3.

Children who were exposed to HIV *in utero* and remained uninfected were found to have altered patterns of cortical structure in early life compared to those who were HIV-unexposed. There was a trend for the cortex to be thicker in HEU compared to HU children across all prefrontal regions with significantly greater cortical thickness in the medial orbitofrontal cortex (mOFC). Language scores, which were lower in HEU compared to HU children, negatively correlated with mOFC thickness in both HEU and HU groups. Using the Baron and Kenny approach, mOFC thickness was found to mediate the relationship between HIV exposure status and poor language outcomes; this was confirmed on structural equation modelling. The findings indicate that HIV exposure may affect cortical maturation of specific brain regions involved in language function, and differences in cortical thickness development in children who are HEU may be a pathway leading to language impairment.

My role in the design, neuroimaging, neurodevelopment, and HIV data collection is listed in Chapters 3 and 7. For this paper, I conceived the manuscript; performed the data analysis with advice from A/Prof Andrea Rehman (statistician), Prof Kirsty Donald, Dr Shantanu Joshi and Prof Katherine Narr (imaging experts); interpreted the results with guidance from my co-authors; drafted the manuscript and incorporated feedback from co-authors and peer reviewers.

List of Figures

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Supplementary material

The supplementary material as detailed in the published article is available at https://doi.org/10.1186/s12916-024-03282-6 and listed in Appendix X.

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Primary Supervisor	Professor Shunmay Yeung		

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RESEARCH ARTICLE

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Association of in utero HIV exposure with child brain structure and language development: a South African birth cohort study

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Abstract

Background There is a growing population of children with in utero HIV exposure who are at risk of poor neurodevelopmental outcomes despite avoiding HIV infection. However, the underlying neurobiological pathways are not understood and neuroimaging studies are lacking. We aimed to investigate the cortical brain structure of children who are HIV-exposed and uninfected (HEU) compared to HIV-unexposed (HU) children and to examine the relationship with neurodevelopment.

Methods The Drakenstein Child Health birth cohort study enrolled pregnant women from a high HIV prevalence area in South Africa with longitudinal follow-up of mother–child pairs. High-resolution magnetic resonance imaging scans from 162 children (70 HEU; 92 HU) were acquired at 2–3 years of age. All HEU children were born to mothers taking antiretroviral therapy. Measures of brain structure (cortical thickness and surface area) in the prefrontal cortex regions were extracted from T1-weighted images and compared between groups using multivariate analysis of variance and linear regression. Child development, assessed using the Bayley Scales of Infant and Toddler Development-III, was correlated with cortical structure, and mediation analyses were performed.

Results Analyses demonstrated an association between HIV exposure and cortical thickness across the prefrontal cortex ($p=0.035$). Children who were HEU had thicker cortices in prefrontal regions, with significantly greater cortical thickness in the medial orbitofrontal cortex (mOFC) bilaterally compared to HU children (3.21 mm versus 3.14 mm, $p=0.009$, adjusted effect size 0.44 [95% CI 0.12 to 0.75]). Estimates held across multiple sensitivity analyses. There were no group differences in cortical surface area. Language scores, which were lower in HEU versus HU children (81.82 versus 86.25, $p=0.011$, effect size −0.44 [95% CI −0.78 to −0.09]), negatively correlated with prefrontal cortical thickness in both groups. Cortical thickness in the mOFC mediated the relationship between HIV exposure and poor language outcomes (Sobel test $p=0.032$).

Conclusions In this cohort study, exposure to HIV during pregnancy was associated with altered cortical structure in early life. Our findings indicate that differences in cortical thickness development in the prefrontal region in children

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who are HEU may be a pathway leading to language impairment. Longitudinal studies are needed to determine the lasting impact.

Keywords HIV, Antiretroviral therapy, Brain structure, Neurodevelopment, Language, Magnetic resonance imaging

Background

Antenatal HIV prevalence remains high in sub-Saharan Africa and in some countries over 20% of children are born to mothers living with HIV [1]. Substantial progress has been made in preventing vertical transmission of HIV with the scale-up of antiretroviral therapy (ART) in pregnancy. Alongside the decline in child HIV infections, the number of children with in utero HIV exposure who remain uninfected is increasing. Currently, there are an estimated 16 million children who are HIV-exposed and uninfected (HEU) worldwide [1]; therefore, any health problems associated with HIV exposure represent notable public health issues.

Children who are HEU have been reported to be at risk of impaired growth and neurodevelopment, particularly those living in low- and middle-income countries (LMICs) [2–7]. Early receptive and expressive language development have been shown to be particularly affected in cohorts from South Africa [8], Botswana [9, 10], and Zimbabwe [11]. A recent meta-analysis found HEU children had poorer expressive language development and gross motor function than HIV-unexposed (HU) children by 2 years of age [12]. Multiple factors may affect brain development during fetal and early life—sensitive periods of brain maturation—and contribute to impaired neurodevelopment in HEU children. These include exposure to HIV and an altered in utero environment, potential neurotoxic effects of ART exposure, and socioenvironmental factors associated with living in an HIV-affected household [3, 13, 14]. However, the neurobiological pathways underlying the association of in utero HIV exposure with poor neurodevelopment remain unclear.

Neuroimaging may be used to investigate neurodevelopmental pathways, in particular whether brain structural alterations underlie cognitive changes, and associations with disease processes [15, 16]. However, evidence is limited in children who are HEU and few studies have reported magnetic resonance imaging (MRI) in this population [17]. The scarce evidence suggests that HIV and/or ART exposure may affect brain development. Altered white matter microstructure in neonates and older children (7–10 years) has been reported in HEU compared to HU children, correlating with neurobehavioural function [18, 19]. Differences in neurometabolites between HEU and HU children have also been found [20, 21]. However, not all results are consistent [22]. In particular, structural imaging studies are lacking, although smaller total grey matter and subcortical brain volumes have been reported in neonates [23, 24]. Separately, individual antiretroviral drugs have been associated with adverse neurodevelopmental outcomes [10, 25], and animal models suggest a potential neurotoxic impact on the neocortex [26]. While qualitative brain imaging abnormalities were described in HEU children exposed to prior zidovudine monotherapy treatment [27], a recent study found that maternal triple ART through gestation may be protective for subcortical structures [24]. However, to our knowledge, no studies of children with in utero HIV and ART exposure have examined cortical surface area and thickness, core components of brain structure that are related to neurocognitive development [28, 29].

The Drakenstein Child Health Study (DCHS) is a South African population-based birth cohort that provides a unique opportunity to examine the brain structure of children who are HEU compared to demographically appropriate HIV-unexposed controls. Building upon our prior findings of increased language delay in HEU children [8], we aimed to compare the cortical neuroanatomy of children who are HEU and HU and to examine the structure–function relationship. We explore two main hypotheses: (1) in utero HIV exposure is associated with altered cortical structure and neurodevelopment at age 2–3 years and (ii) atypical patterns of structural brain development mediate the relationship between HIV exposure and neurodevelopmental function.

Methods

Study design and participants

This is a prospective neuroimaging study nested within the DCHS, a longitudinal birth cohort in a peri-urban area of the Western Cape, South Africa [30, 31]. The population is characterised by high levels of poverty and an antenatal HIV prevalence of 21% [32]. Mothers were enrolled between 2012 and 2015 from two public sector primary health care clinics at 20–28 weeks' gestation while attending routine antenatal appointments. Eligibility criteria included age 18 years or older and intention to remain in the area attending one of the two clinics. Written informed consent was obtained at enrolment, and mothers are reconsented annually.

A sub-group of children aged between 2 and 3 years participated in the neuroimaging sub-study and were invited for an MRI scan between January 2016 and September 2018 following a small pilot [33]. Methods for

child recruitment and neuroimaging are described in full elsewhere [33]. Briefly, children from the DCHS were eligible for neuroimaging if they resided in the study area, were aged 2–3 years, and did not have the following exclusion criteria: (i) medical comorbidity (genetic syndrome, neurological disorder, congenital abnormality); (ii) gestation < 36 weeks; (iii) Apgar score < 7 at 5 min; (iv) neonatal intensive care admission; (v) maternal use of illicit drugs during pregnancy; (vi) MRI contraindications; (vii) child HIV infection. All children with MRIs as neonates were invited for a scan at age 2–3 years, and additional children were selected for MRI to ensure adequate representation of risk factor exposure (including maternal HIV) along with a randomly selected comparison group [33]. Written informed consent was obtained from the parent/guardian at the neuroimaging visit.

Study procedures

Mothers received routine HIV testing during pregnancy and the postnatal period following the Western Cape Prevention of Mother-to-Child Transmission (PMTCT) of HIV guidelines [34]. All HEU children had HIV-negative status confirmed through testing at 6 weeks using polymerase chain reaction (PCR) tests and at 9 and 18 months and post-cessation of breast-feeding using PCR, enzyme-linked immunosorbent assays, or rapid antibody testing as appropriate. Pregnant women living with HIV who were diagnosed before May 2013 received triple-drug ART or zidovudine monotherapy from 14 weeks' gestation with nevirapine at delivery, based on WHO clinical stage and CD4 cell count, while those diagnosed after that point received triple-drug ART for life. HIV-exposed children received nevirapine prophylaxis alone or combined with zidovudine. HU children were defined as children born to HIV-uninfected mothers. Data on maternal ART use, infant prophylaxis, maternal CD4 cell count, and viral load data were collected from interviews, clinical notes, and the online National Health Laboratory Service system.

Sociodemographic data were collected at a baseline assessment during the third trimester of pregnancy using structured interviews and standardised questionnaires, and maternal smoking and alcohol use during pregnancy were also assessed [30, 31]. Maternal smoking was measured by self-report. Maternal alcohol use was assessed and quantified using the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) and retrospectively collected data on moderate-severe alcohol use in pregnancy forming a dichotomous measure [35]. Birth anthropometry measures were abstracted from hospital records. Weight and head circumference were measured at the scan and recorded using a standard protocol. Feeding data, including exclusive breastfeeding duration, were reported by mothers across multiple visits [32].

Neurodevelopmental assessment

The Bayley Scales of Infant and Toddler development, third edition (BSID-III), was used to assess cognitive, language, and motor outcomes of children [35]. Two trained and experienced local assessors administered the BSID-III offering language prompts in the child's preferred language, blinded to HIV exposure status. Assessors were monitored by a Paediatric Neurodevelopmental specialist to ensure reliability, accuracy, and standardised data collection. Age-adjusted composite scores were generated using normative values from a US reference population with a mean of 100 and a standard deviation of 15 [36]. Composite scores are standardised allowing comparison across ages and settings, and these have been validated in a South African setting [37]. Referral into appropriate clinical pathways for children with developmental delay was arranged.

Neuroimaging

Image acquisition

Children were scanned during natural, non-sedated sleep to limit motion and scans were scheduled during typical sleep schedules using a child-friendly approach [33]. Once children were in a deep sleep, they were carefully positioned in the scanner with ear protection and stabilising cushions to reduce head movement during scans. High-resolution structural T1-weighted MR images were taken using a 3-Tesla Siemens Skyra 70-cm bore whole-body MRI scanner (Erlangen, Germany) at the Cape Universities Brain Imaging Centre, Groote Schuur Hospital, with a 32-channel head coil. A 3D MEMPRAGE (Multi-Echo Magnetization Prepared Rapid Acquisition Gradient Echo) sequence was used in sagittal orientation with the following image parameters: repetition time = 2530 ms; echo time = 1.69, 3.54, 5.39, 7.24 ms; inversion time = 1100 ms; flip angle = 7.0°; voxel size $1.0 \times 1.0 \times 1.0$ mm^3; field of view = $224 \times 224 \times 176$ mm; 176 slices.

Image processing

Images were processed with FreeSurfer version 6.0 software [38], at the local supercomputing cluster at the Centre for High Performance Computing (CHPC, Cape Town). The cortex was parcellated into regions according to the Desikan-Killiany atlas [39] and measures of cortical structure (cortical thickness, mm, and surface area, mm^2) were extracted for analysis (see Additional file 1: Text S1) [39–43].

Quality control

All structural sequences were reviewed by a radiologist blinded to HIV exposure status for incidental findings. Abnormal reports were referred for follow-up through appropriate local clinical pathways. Each scan was visually inspected for motion artefacts and for errors in segmentation processing following the standardised ENIGMA protocol [44].

Region selection

Given reported neurodevelopmental impairment in children who are HEU [12], we hypothesised regions of the prefrontal cortex may be affected. Therefore, we conducted targeted analyses, expanding on prior exploratory findings showing an association between the frontal region and neurocognitive function in the early years [33]. All regions in the prefrontal cortex were selected a priori as they were determined to be biologically plausible areas due to their critical role in neurocognitive functioning and vulnerability to environmental exposures (further details may be found in Additional file 1: Text S1) [8, 16, 45–50]. For each participant, the mean values of the left and right hemispheres were used for analyses of each measure (cortical thickness, cortical surface area). The prefrontal regions are illustrated in Fig. 1, along with the components of cortical brain structure.

Statistical analysis

Sociodemographic group differences were assessed using unpaired *t*-tests, chi-squared, or Fisher's exact tests as appropriate between children who are HEU compared to HU. Comparisons were also made between the neuroimaging subgroup and the full DCHS cohort to assess generalisability.

To investigate group differences in cortical structure by HIV exposure, we first used multivariate analysis of variance to examine for a group-by-region effect in cortical surface area and cortical thickness separately. Pending a group-by-region effect, independent linear regression models were constructed to compare cortical structure in prespecified regions of interest (ROIs) between HEU and HU children. Partially adjusted multivariable linear

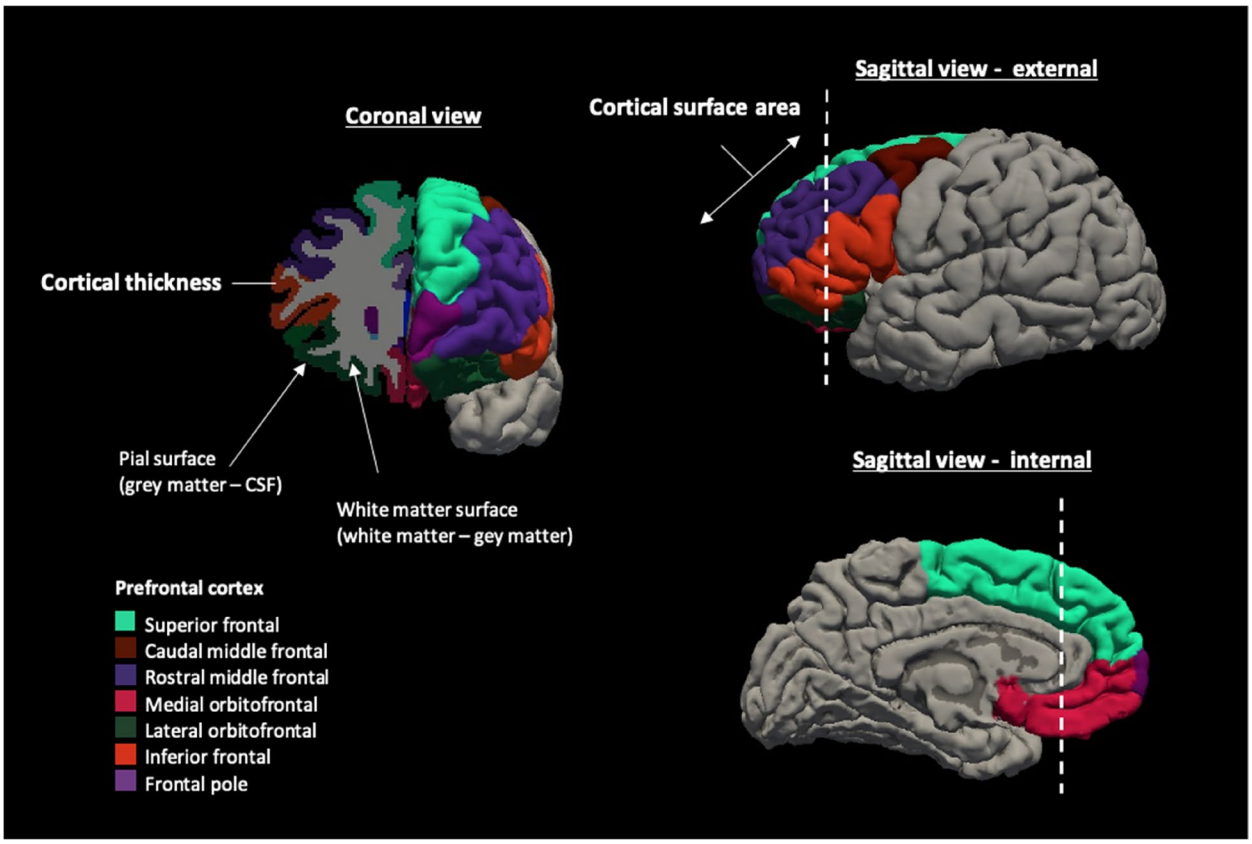

Fig. 1 Schematic of the prefrontal cortex regions and the structural metrics of cortical brain structure. Cortical brain structure represents cortical thickness and cortical surface area. Cortical thickness describes the thickness of the layers of the cerebral cortex and is calculated as the distance from the white matter surface (white matter-grey matter boundary) to the pial surface (grey matter-CSF boundary). Cortical surface area and cortical thickness were calculated for each region. Regions visualised using a FreeSurfer template brain. Abbreviation: CSF, cerebrospinal fluid

models were created including child sex and child age as a priori confounders of interest [51]. Fully adjusted multivariable linear regression models were then created including additional confounders identified from the literature using a directed acyclic graph (DAG) including household income, maternal education, and age [8, 52]. We did not include characteristics that may be on the causal pathway. Where a significant association ($p<0.05$) was identified, we examined each hemisphere separately using the same model. Mean differences and standardised effect sizes were reported using Cohen's *d*. Normality of residuals and homogeneity of variance were checked in each model using quantile–quantile plots and scatterplots. Sensitivity analyses were performed to ensure our results were not impacted by alcohol use in pregnancy. Furthermore, we conducted restricted analyses of (i) the site where the majority of HEU children attended and (ii) limiting the HEU group to those exposed to the same maternal first-line ART regimen.

Neurodevelopmental outcomes were compared between groups using multivariable regression models described above, including cognitive, language, and motor composite scores as dependent variables. We then calculated Pearson's correlation coefficients between cortical structure and neurodevelopmental outcomes for all ROIs in neurodevelopmental domains that showed a difference between HEU and HU groups ($p<0.05$). We report correlation coefficients in the full sample and stratified by HIV exposure. We also calculated partial correlation coefficients adjusting for covariates as above. To explore evidence for effect modification by HIV exposure in regions with a significant correlation ($p<0.05$), a linear regression model was fitted with neurodevelopment indices as dependent variables and the interaction between group and cortical variables.

Finally, we conducted a mediation analysis to test the hypothesis that associations between HIV exposure and neurodevelopmental outcomes are mediated by cortical brain structure. We applied the Baron and Kenny approach [53] that uses sequential regression analyses to test for mediation (see Additional file 1: Text S1). Models were adjusted for potential confounding variables identified a priori. We confirmed the results using structural equation modelling [54]. Statistical analyses were performed using STATA 14.2 (StataCorp Inc, College Station, TX, USA). $P<0.05$ (two-tailed) was considered statistically significant.

Results

Demographics

A total of 1143 infants were born to 1137 women in the DCHS between May 2012 and September 2015. Two children were diagnosed with HIV infection and were not included in this analysis. Cohort retention was high, with 1000/1141 (87.6%) children in follow-up at 2 years. A sub-group of 216 children attended for MRI between January 2016 and September 2018 aged 2–3 years, following a small pilot (Fig. 2). High-resolution T1-weighted images were included for 162/216 (75%) children (70 HEU, 92 HU) excluding those children who did not sleep or scans that did not reach quality thresholds due to movement. Children in the neuroimaging sub-group were representative of the full cohort in socioeconomic variables (Additional file 1: Table S1).

Mothers and children in the two groups had similar demographic characteristics (Table 1). However, HEU children were born to mothers who were older, and a greater proportion were seen at Mbekweni clinic compared to TC Newman clinic. Children who were HEU had a trend for lower head circumference measurements at birth ($p=0.06$), although this was not sustained at 2 years and other anthropometric measurements were comparable. Of the women living with HIV, 98.6% were taking triple-drug ART during pregnancy (97.1% non-nucleoside reverse transcriptase inhibitor-based ART; 1.4% protease inhibitor-containing ART) and 1.4% zidovudine monotherapy (Additional file 1: Table S2). Approximately half (39/70; 55.7%) of the mothers initiated ART during pregnancy. Median maternal CD4 was 476 cells/mm^3, and most women with a viral load result had undetectable levels (77.2%) during pregnancy. The majority of children received postnatal prophylaxis with nevirapine alone (80.0%), versus nevirapine and zidovudine in the remainder.

Neuroanatomy

Total intracranial volume was similar between child HEU (1208 cm^3) and HU (1219 cm^3) groups ($p=0.586$). Multivariate group analysis showed a significant group-by-region effect for HIV exposure on cortical thickness measurements across the prefrontal cortex [F (7, 154) = 2.22, $p=0.035$] but not for cortical surface area [F (7, 154) = 0.18, $p=0.989$].

We therefore conducted further analyses on cortical thickness. Compared to HU children, HEU children had thicker cortices across all prefrontal cortex regions (Fig. 3). The model of the medial orbitofrontal cortex (mOFC) regions was statistically significant (Table 2). This held after adjusting for potential confounding variables with a moderate effect size (3.21 mm [HEU] versus 3.14 mm [HU], Cohen's *d* 0.44 [95% confidence interval, CI 0.12 to 0.75], $p=0.009$) (Table 2; Fig. 3). Post hoc tests demonstrated both hemispheres contributed to the overall mOFC effect (left mOFC effect size 0.36 [0.04 to 0.67]; right mOFC effect size 0.44 [0.12 to 0.75]).

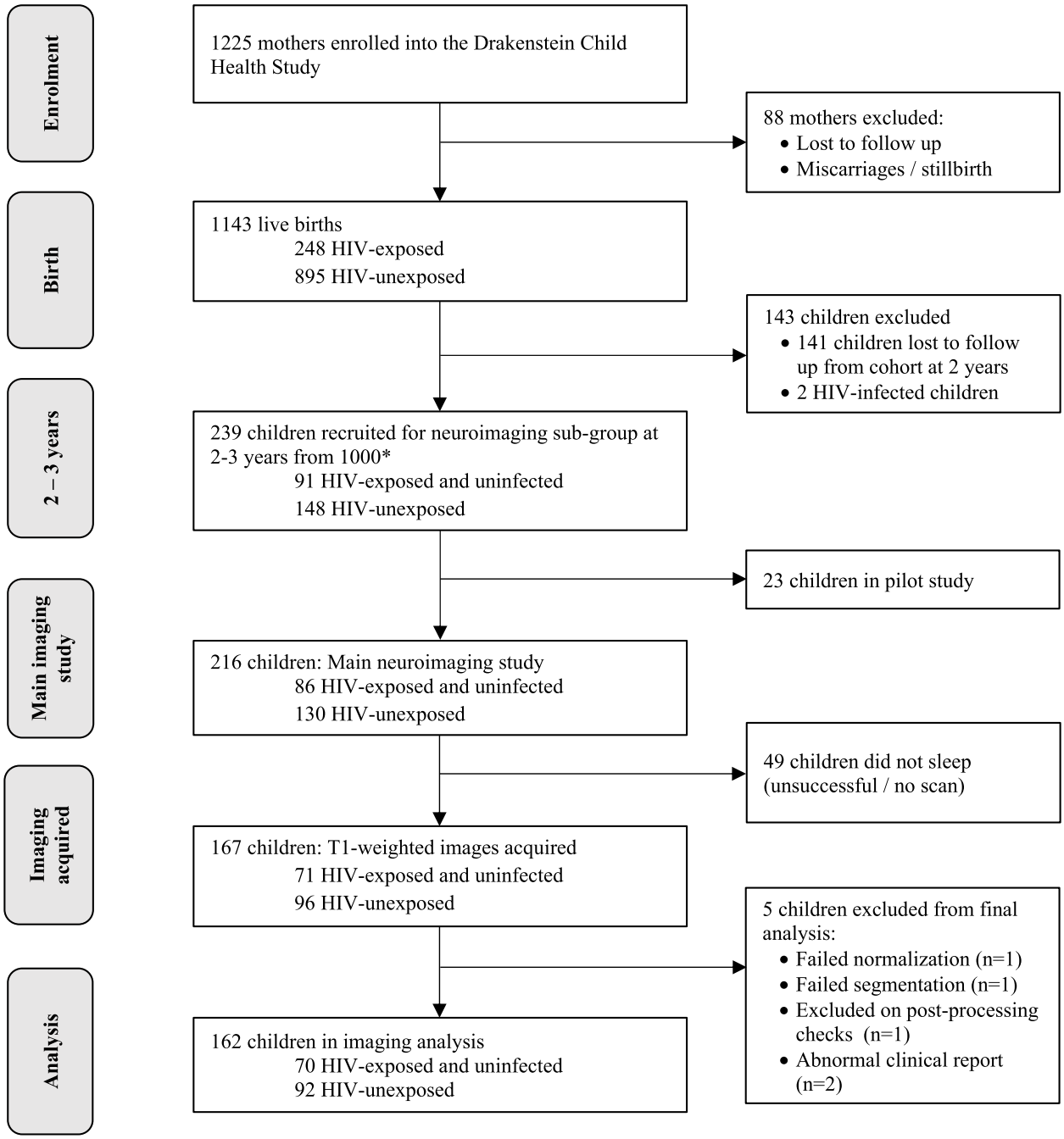

Fig. 2 Drakenstein Child Health Study cohort flow chart of children with neuroimaging by HIV exposure. *Selection criteria for neuroimaging are fully described in methods. Inclusion criteria are as follows: (i) currently active in the cohort; (ii) residing in the study area; (iii) child aged 2–3 years. Exclusion criteria are as follows: (i) medical comorbidity (genetic syndrome, neurological disorder, or congenital abnormality); (ii) gestation < 36 weeks; (iii) low Apgar score (< 7 at 5 min); (iv) neonatal intensive care admission; (v) maternal use of illicit drugs during pregnancy; (vi) MRI contraindications; (vii) child HIV infection

Our findings persisted across sensitivity analyses adjusting for self-reported alcohol use during pregnancy. We performed an analysis restricted to children from Mbekweni clinic to address between-site differences which revealed similar results, as did limiting the HEU group to those exposed to the same first-line regimen (efavirenz, emtricitabine/lamivudine, tenofovir) (Additional file 1: Table S3 and Table S4).

Table 1 Sociodemographic and clinical characteristics by HIV exposure

Variable	Total $N=162$	HEU children $N=70$	HU children $N=92$	*P*-value
Sociodemographic characteristics				
Child age at scan, months	34.1 (1.7)	33.8 (1.8)	34.3 (1.7)	0.096
Sex				
Female	68 (42.0%)	24 (34.3%)	44 (47.8%)	
Male	94 (58.0%)	46 (65.7%)	48 (52.2%)	0.084
Site				
TC Newman	48 (29.6%)	5 (7.1%)	43 (46.7%)	
Mbekweni	114 (70.4%)	65 (92.9%)	49 (53.3%)	< 0.001*
Monthly household income (ZAR)				
< R1000 (< ~ $75)	51 (31.5%)	24 (34.3%)	27 (29.4%)	
> R1000	111 (68.5%)	46 (65.7%)	65 (70.7%)	0.503
Maternal education				
Any secondary	108 (66.7%)	51 (72.9%)	57 (62.0%)	
Completed secondary	54 (33.3%)	19 (27.1%)	35 (38.0%)	0.145
Maternal employment status (employed)	44 (27.2%)	17 (24.3%)	27 (29.4%)	0.473
Maternal age at birth, years	28.0 (5.6)	29.6 (4.9)	26.8 (5.8)	0.002*
Maternal smoking during pregnancy	32 (19.8%)	9 (12.9%)	23 (25.0%)	0.054
Maternal alcohol use during pregnancy	24 (18.2%)	7 (13.5%)	17 (21.3%)	0.257
Duration of exclusive breastfeeding (months)	1.8 (1.8)	1.6 (2.1)	2.0 (1.5)	0.097
Anthropometry				
Birthweight, kg	3.1 (0.6)	3.0 (0.6)	3.1 (0.6)	0.291
Birth head circumference, cm	33.6 (2.0)	33.3 (1.9)	33.9 (2.0)	0.061
Weight at scan, kg	13.9 (1.9)	13.9 (2.0)	13.8 (1.9)	0.629
Head circumference at scan, cm	49.8 (1.8)	49.8 (1.8)	49.7 (1.8)	0.969
Neuroanatomical variables				
Total intracranial volume, mean (SD), cm^3	1214 (118)	1208 (116)	1219 (119)	0.586
Maternal and child HIV variables				
Maternal CD4 in pregnancy, median (IQR) (cells/mm^3)		476 (344—677)		-
< 350 cells/mm^3		17 (27.9%)		-
350–500 cells/mm^3		16 (26.2%)		-
≥ 500 cells/ mm^3		28 (45.9%)		-
Maternal Viral load (VL) in pregnancy				
Lower than detectable (< 40 copies/mL)		44 (77.2%)		-
VL detectable (≥ 40–1000 copies/mL)		7 (12.3%)		-
Virally unsuppressed (> 1000 copies/mL)		6 (10.5%)		-
Timing of antiretroviral drug initiation				
Before conception		31 (44.3%)		-
During pregnancy		39 (55.7%)		-
Antiretroviral regimen during pregnancy				
Monotherapy with AZT [zidovudine]		1 (1.4%)		-
2 NRTIs + NNRTI [1st line]		68 (97.1%)		-
2 NRTIs + PI [2nd line]		1 (1.4%)		-
Infant prophylaxis				
NVP [nevirapine] alone		56 (80.0%)		-
NVP + AZT		14 (20.0%)		-

Data are *N* (%), mean (SD), or median (IQR). Continuous variables were compared with unpaired *t*-tests; categorical variables were compared with chi-squared tests. *$p < 0.05$. Percentages are cited among those with non-missing values. Missing data: alcohol ($n=30$); birthweight ($n=1$); head circumference at birth ($n=1$) and at scan ($n=1$); maternal CD4 ($n=9$); maternal viral load ($n=13$). The lowest maternal CD4 within 1 year prior to birth and 3 months post-birth was used to refle t maternal immunosuppression in pregnancy and maximise sample numbers. Maternal viral load was measured during pregnancy; where there was more than one result, the highest viral load was taken. Of the NRTI + 2NNRTIs, 64 mothers were taking efavirenz, emtricitabine/lamivudine, and tenofovir. *Abbreviations*: *HEU*, HIV-exposed and uninfected; *HU*, HIV-unexposed; *VL*, viral load; *NNRTI*, non-nucleoside reverse-transcriptase inhibitor; *NRTI*, nucleoside reverse transcriptase inhibitor; *PI*, protease inhibitor; *NVP*, nevirapine; *AZT*, zidovudine

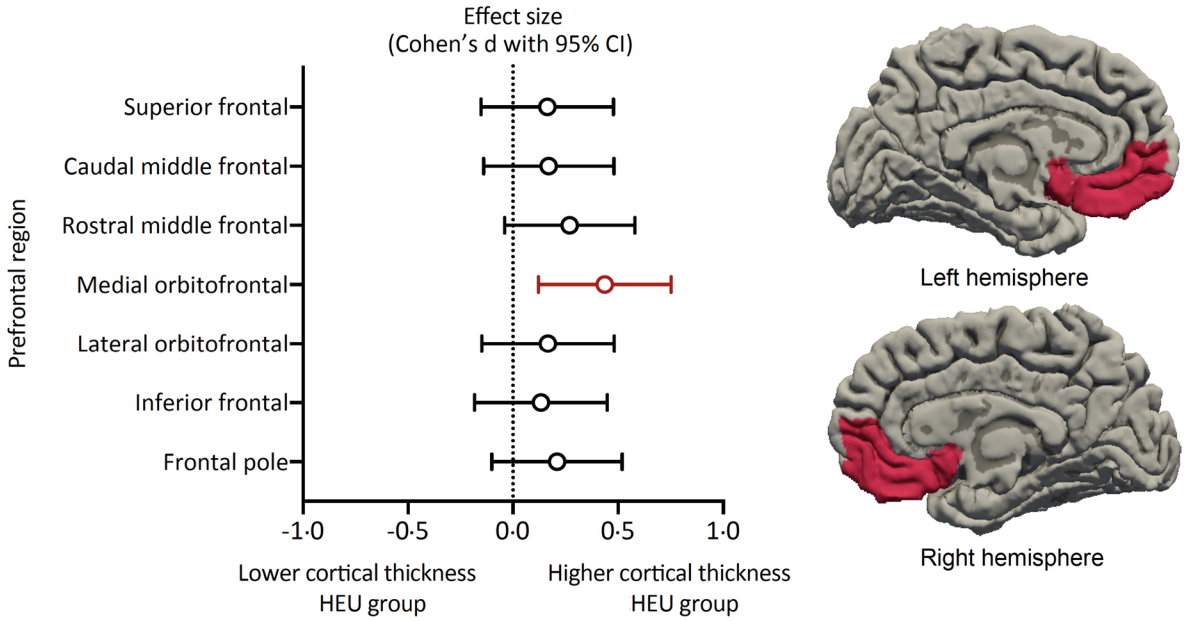

Fig. 3 Associations of cortical thickness in anatomical regions of the prefrontal cortex with HIV exposure. Left panel: Figure displaying Cohen's *d* effect sizes and 95% confidence intervals for cortical thickness differences between HEU and HU children across the prefrontal cortex regions. Effect sizes are shown after correction for child age and sex, household income, maternal age, and education. Positive values indicate higher cortical thickness in HEU children. Right panel: Regions with significantly increased cortical thickness in HEU children are highlighted in red on left and right medial views. Abbreviation: HEU, children who are HIV-exposed and uninfected

Table 2 Adjusted mean differences in cortical thickness across the prefrontal cortex by HIV exposure groups

Prefrontal cortex regions	Cortical thickness (mm)		Minimally adjusted model[a]			Full adjusted model[b]		
	HEU Mean (SD) (*n* = 70)	HU Mean (SD) (*n* = 92)	Mean difference (95% CI)	*p*-value	Effect size	Mean difference (95% CI)	*p*-value	Effect size
Superior frontal	3.31 (0.16)	3.30 (0.16)	0.01 (−0.04 to 0.06)	0.607	0.08 (−0.23 to 0.39)	0.03 (−0.03 to 0.08)	0.321	0.16 (−0.15 to 0.48)
Caudal middle frontal	2.96 (0.16)	2.93 (0.15)	0.02 (−0.03 to 0.07)	0.351	0.15 (−0.16 to 0.46)	0.03 (−0.02 to 0.08)	0.310	0.17 (−0.14 to 0.48)
Rostral middle frontal	2.97 (0.12)	2.95 (0.13)	0.02 (−0.02 to 0.06)	0.319	0.16 (−0.15 to 0.47)	0.03 (−0.01 to 0.07)	0.102	0.27 (−0.04 to 0.58)
Medial orbito-frontal	3.21 (0.21)	3.14 (0.19)	0.08 (0.01 to 0.14)	0.016*	0.39 (0.07 to 0.70)	0.09 (0.02 to 0.15)	0.009*	0.44 (0.12 to 0.75)
Lateral orbito-frontal	3.27 (0.14)	3.26 (0.15)	0.01 (−0.04 to 0.06)	0.648	0.07 (−0.24 to 0.39)	0.02 (−0.02 to 0.07)	0.318	0.17 (−0.15 to 0.48)
Inferior frontal	3.15 (0.13)	3.15 (0.13)	0.00 (−0.04 to 0.04)	0.923	0.02 (−0.30 to 0.33)	0.02 (−0.03 to 0.06)	0.412	0.13 (−0.18 to 0.45)
Frontal pole	3.54 (0.27)	3.51 (0.30)	0.04 (−0.05 to 0.13)	0.386	0.14 (−0.17 to 0.45)	0.06 (−0.03 to 0.15)	0.212	0.21 (−0.10 to 0.52)

Multiple linear regression estimates for HIV exposure on cortical thickness. *$p < 0.05$. [a]Adjusted for child age and sex. [b]Adjusted for child age and sex, household income, maternal age, and education. Cortical thickness (mean of left and right hemispheres), mean differences (regression coeffici ts minimally and fully adjusted in multiple regression models), *p*-values, and effect sizes are presented. Effect sizes were calculated using Cohen's *d* with associated 95% confiden e intervals. Residuals were assessed for each model using quantile–quantile plots and scatterplots and were normally distributed. A positive mean difference estimate indicates that HIV exposure is associated with thicker cortex in that region. Post hoc, the regions with a significa t difference were assessed bilaterally in fully adjusted models: medial orbitofrontal cortex (left hemisphere) effect size 0.36 (0.04 to 0.67) and (right hemisphere) effect size 0.44 (0.12 to 0.75). *Abbreviations: HEU*, HIV-exposed and uninfected; *HU*, HIV-unexposed

Neurodevelopmental outcomes

Among children in the neuroimaging subgroup with neurodevelopmental data ($n=146$), HEU children had lower composite language scores on BSID-III compared to HU children in minimal and fully adjusted analyses (81.82 versus 86.25, $p=0.011$, adjusted Cohen's d effect size −0.44 [−0.78 to −0.09]), while cognitive and motor composite scores were similar (effect size −0.21 [−0.54 to 0.12] $p=0.228$ and effect size −0.09 [−0.43 to 0.24] $p=0.602$ respectively). Supplementary analyses with raw scores showed that differences in both receptive and expressive language were evident (Additional file 1: Table S5).

Neuroanatomical regional associations with language function

Language development was negatively correlated with cortical thickness in multiple regions of the prefrontal cortex, most strongly with the mOFC ($r=-0.31$, $p=0.0002$) (Additional file 1: Table S6). After adjusting for the full covariate set, only the correlation between language and mOFC thickness remained significant ($r=-0.27$, $p=0.002$), and this was seen bilaterally (left mOFC: $r=-0.22$, $p=0.012$; right mOFC $r=-0.28$, $p=0.001$). When stratified by HIV exposure, correlations with mOFC thickness remained significant, and we found a stronger negative correlation in children who were HEU ($r=-0.35$, $p=0.008$) compared to HU ($r=-0.23$, $p=0.038$) (Fig. 4; Additional file 1: Table S6). However, on modelling the interaction effect, there was no effect modification by HIV exposure for the association between mOFC thickness and language outcomes ($p=0.759$).

Mediation analyses

Given the identified associations between in utero HIV exposure with cortical thickness and language outcomes, we conducted a mediation analysis. We found that increased cortical thickness in the mOFC mediated the observed association between HIV exposure and poor language outcomes through the Baron and Kenny approach [53] (significance testing of the indirect effect using the Sobel test: $p=0.032$) (Fig. 5). We present estimates of the total and direct effects of HIV exposure on language development, alongside estimates of the indirect (mediated) effect that may be explained by the influence of HIV exposure on [adjusted] mOFC thickness. The proportion mediated through increased mOFC thickness was estimated to be 35%. This was further supported by structural equation modelling (Additional file 1: Table S7).

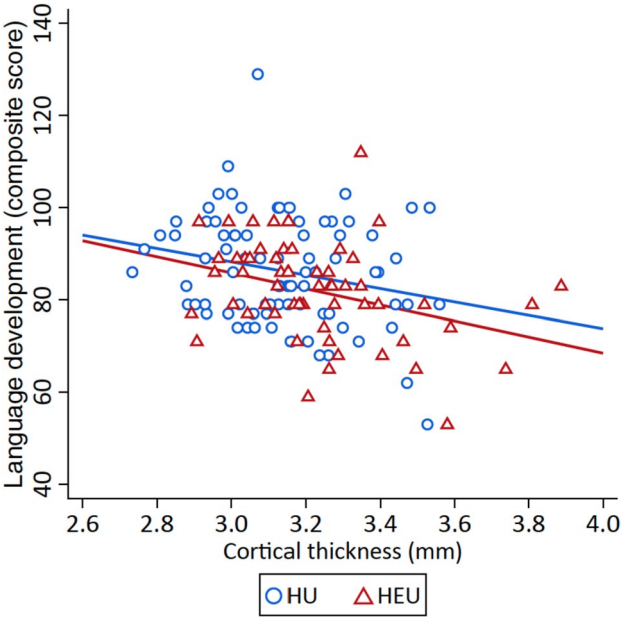

Fig. 4 Linear regression of child language development by mOFC cortical thickness, stratified by HIV exposure. The relationship of child composite language score (BSID-III) with medial orbitofrontal region cortical thickness (mm) for HEU and HU children with line of best fit, $p<0.05$. The direction of the correlation is negative, i.e. lower language scores are associated with a thicker cortex. Abbreviations: HEU, children who are HIV-exposed and uninfected; HU, children who are HIV-unexposed; mOFC, medial orbitofrontal cortex

Discussion

In this South African birth cohort, in utero HIV exposure was associated with altered cortical thickness in the prefrontal cortex at age 2–3 years. Language scores, which were lower in children who were HEU compared to HU, negatively correlated with cortical thickness in both groups. Overall, cortical thickness differences in the medial orbitofrontal region mediated approximately one third of the relationship between HIV exposure and language outcomes. Our results suggest that underlying changes in cortical brain structure may be one pathway leading to language impairment seen in children who are HEU, and specific prefrontal region functions may be disrupted at this age point.

Our findings that children with in utero HIV exposure had greater cortical thickness across all regions of the prefrontal cortex and significant cortical thickness differences in the mOFC bilaterally are novel. Few neuroimaging studies have been conducted in children who are HEU, and to our knowledge, cortical thickness and surface area have not been previously described. However, our results are consistent with reports using other imaging modalities showing that HIV and/or ART exposure may impact brain development. Previous studies have found lower subcortical volumes in early infancy

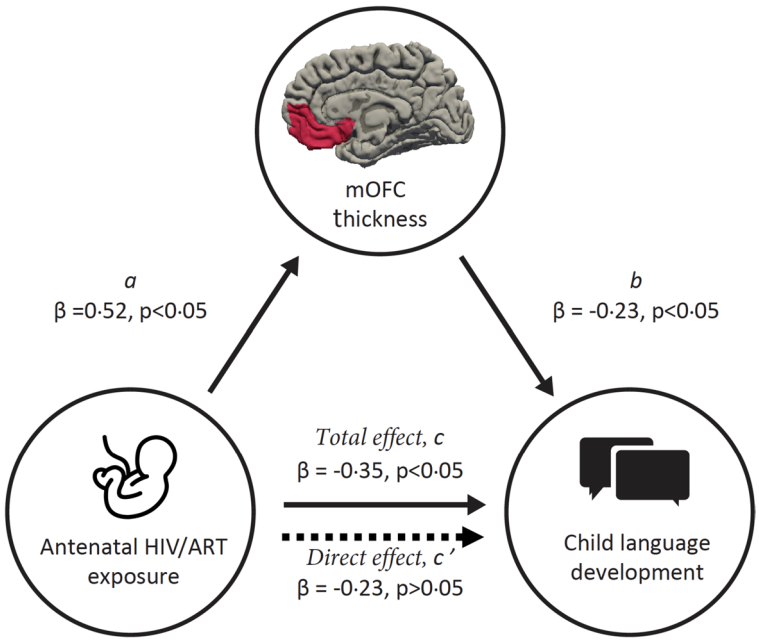

Fig. 5 Illustration of mediation paths: mOFC thickness as a mediator between HIV exposure and child language. Estimates of the total (path *c*), direct (path *c'*), and indirect (path *ab*; mediated through the influence on structural brain development) effects of HIV exposure on child language. The proportion of the total effect of HIV exposure on child language mediated via mOFC thickness ≈ 35%, Sobel test $p = 0.032$. Results are displayed as standardised β regression coefficients adjusted for child age and sex, maternal age, education, and household income. Complete case analysis used $N = 138$ ($N = 81$ HEU, $N = 57$ HU). Abbreviations: mOFC, medial orbitofrontal cortex; HEU, HIV-exposed and uninfected; HU, HU-unexposed

in HEU compared to HU children [23, 24], differences in neurometabolites at 7 years [20], and altered white matter microstructure in neonates and older children associated with neurobehavioural function [17–19]. A limited number of studies of children with HIV infection have examined cortical modelling. Altered cortical thickness in 10–11-year-olds has been reported, including higher thickness in frontal and cingulate regions compared to controls [46].

Cortical thickness is a key component of brain structure, representing the number of neurons, synapses, glial, and dendritic processes connecting the layers of the neocortex [55]. Cortical thickness develops along an inverted U-shaped trajectory, increasing initially, peaking around 1–2 years, and then steadily decreasing through childhood as maturation occurs [16, 56]. The biological basis for the natural maturational thinning across the cortex is thought to reflect neuronal and synaptic pruning or myelination, leading to the formation of more organised and refined neural circuits [15, 56]. HEU children may therefore be demonstrating delayed cortical maturation or disrupted pruning or myelination at this early age. While the clinical significance of this remains to be determined, studies have found profiles of delayed cortical maturation may be associated with mood disorders [57], attention-deficit/hyperactivity disorder [58], and autism spectrum disorders [59], as well as being linked to other exposures including alcohol [60, 61] and infections such as cytomegalovirus infection (CMV) [62]. The process of cortical maturation is dynamic and there is substantial regional heterogeneity in the cortical thickness trajectory. Orbitofrontal regions are reported to peak the earliest (around 1 year) and decrease the fastest [15, 56]. We found that the medial orbitofrontal regions are the most affected in HEU children. This may reflect region-specific vulnerability or differences in the timing of development. Alternatively, the mOFC may be the earliest affected and other regions may become evident with time, highlighting the importance of longitudinal follow-up and serial imaging.

We found no evidence of differences in cortical surface area between HEU and HU children in our sample. Cortical thickness and surface area have heterogeneous temporal and regional patterns of development [15, 63], and while cortical thickness has been found to be influenced by environmental factors, surface area has stronger genetic links which may explain our findings [45]. Further, cortical thickness is largely determined in early life [45], whereas surface area is estimated to be 70% at 1 year and continues to increase [15]; therefore, changes may manifest later.

In this neuroimaging sub-group, HIV exposure was associated with lower language scores. Although there is heterogeneity across the broader literature with respect to neurodevelopment [12, 64], these findings are consistent with studies from multiple settings reporting worse language outcomes in HEU than in HU children [9, 11, 65], suggesting early language development may be at risk in children who are HEU. Further, we demonstrate that language development was negatively correlated with cortical thickness across multiple prefrontal regions. The most robust correlation was seen with the mOFC among both HEU and HU groups with a moderate effect size. It is well-established that the dynamic development of cognitive abilities parallels cortical maturation in early childhood [16, 28]. Our results are consistent with child development studies that have shown earlier cortical thinning in childhood is linked to the development of a more efficient network and better cognitive and language outcomes [28, 52], including verbal fluency [66].

Building on these findings, our mediation analysis demonstrated that approximately one third of the observed association between HIV exposure and language may be explained through an impact on mOFC cortical thickness. The results suggest that altered cortical maturation in frontal regions may partly underlie the reported language deficits. In early life, there is evidence to suggest a more widespread network of regions underly language function compared to later life [67, 68], with core input from higher-order centres, including the prefrontal cortex [69], until it becomes more automated [70]. The mOFC has previously been implicated in goal-directed behaviour, executive function, and reward processing [71, 72]. In the first few years, the rapid development and integration of sensory-motor and cognitive pathways in frontal regions which underly these functions may also contribute to language [73]. This has implications for later language functions such as sentence completion and story comprehension which have been associated with the mOFC [71].

Overall, we hypothesise that processes governing brain structural development in regions that support neurocognition may be disrupted in children who are HEU, impacting language outcomes. Several mechanisms may potentially impact cortical maturation processes of myelination and pruning related to perinatal HIV exposure including neuroinflammation, ART neurotoxicity, neurological infections such as CMV, and socioenvironmental factors [14, 16]. Given that 97% of mothers were on the same ART regimen (efavirenz, tenofovir, and emtricitabine) and most had suppressed viral loads, it is difficult to disentangle the contributions of HIV and ART. However, previous studies have highlighted an association between efavirenz and poorer receptive language [10] and microcephaly [25], and these need to be explored further. Separately, evidence from clinical and pre-clinical studies indicates that the immune system plays a critical role in brain development [74] including synaptic pruning [75], and maternal immune activation in pregnancy has been shown to alter prefrontal cortex morphology in particular [76]. Future studies should consider the impact of maternal immune function on brain development in this vulnerable group. Furthermore, given cortical structure mediated one third of the HIV-language relationship, ongoing exploration into the other contributing biological processes and mechanistic pathways linking HIV-specific and universal risk factors to HEU child neurodevelopmental outcomes is warranted.

There are several strengths of this study that add to the existing literature. This is the largest neuroimaging study to date to compare cortical brain structure between HEU and HU children during a critical period of brain growth. We explored cortical thickness and surface area in HEU children for the first time, the core components of cortical brain structure. We performed comprehensive neuroimaging and neurodevelopmental assessments blinded to HIV status minimising the risk of bias. Furthermore, this study was conducted amongst a well-characterised sample of HEU children representative of other high HIV-burden countries [77], with demographically appropriate controls, expanding generalisability from previous work.

There are limitations of the study that should be considered. Firstly, although our sample size was reasonably large for a neuroimaging study, we note that greater sample sizes are needed to withstand multiple comparisons across whole-brain analyses. We acknowledge the complexity and rapidly evolving nature of brain development, and further work is needed to explore other brain regions, including temporal, parietal, and subcortical structures, to understand wider network effects. Secondly, inherent challenges to MRI in young children mean we cannot rule out selection bias. However, mitigating these concerns, we established that sociodemographic characteristics were similar between children with and without imaging. Thirdly, as an observational study, this analysis does not establish causality. We measured neurodevelopment as standardised scores, with predictive validity across ages [78, 79], indicative of the child neurodevelopmental trajectory. However, longitudinal analyses are needed to confirm the relationship between structural and functional changes in children who are HEU at older ages. While the BSID-III has been validated for use in South Africa [37, 80], using a tool that is standardised in other populations is a limitation and there are reliability concerns regarding the use of US-normed data which may affect generalisability. We include a control group and share raw scores to add validity to our outcomes. Further

standardisation is needed in SSA settings using contextually appropriate norms. Finally, although estimates held across multiple sensitivity analyses, other unmeasured confounding variables such as CMV infection may have resulted in residual bias, and there are likely multifactorial causal pathways. Further research would benefit from investigating potential mechanisms, in particular exploring the association with ART, including newer dolutegravir-based regimens which are now first-line treatment for HIV in pregnancy.

Conclusions

In conclusion, we found altered patterns of cortical structure in children with in utero HIV exposure compared to demographically similar children without exposure at 2–3 years. Cortical thickness in the medial orbitofrontal cortex mediated the association between in utero HIV exposure and poor language outcomes. The findings suggest that HIV exposure may affect the maturation of prefrontal brain regions with implications for neurodevelopmental function. This has public health significance for the growing HEU population given brain development in early childhood is critical for long-term cognitive outcomes. However, it remains to be established whether these alterations persist, highlighting the need for ongoing neurodevelopmental surveillance and further studies to examine trajectories of brain maturation and underlying mechanisms.

Abbreviations

ART	Antiretroviral therapy
ASSIST	Alcohol, Smoking and Substance Involvement Screening Test
BSID-III	Bayley Scales of Infant and Toddler Development, 3rd edition
CHPC	Centre for High Performance Computing
CI	Confidence interval
CMV	Cytomegalovirus
DAG	Directed acyclic graph
DCHS	Drakenstein Child Health Study
HEU	HIV-exposed uninfected
HIV	Human immunodeficiency virus
HU	HIV-unexposed
LMIC	Low- and middle-income countries
MEMPRAGE	Multi-Echo Magnetization Prepared Rapid Acquisition Gradient Echo
mOFC	Medial orbitofrontal cortex
MRI	Magnetic resonance imaging
ROI	Region-of-interest
PCR	Polymerase chain reaction
PMTCT	Prevention of mother-to-child transmission
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-024-03282-6.

Additional file 1: Text S1. Detailed methods for image processing and analysis**. Table S1.** Comparison of study demographics of children with imaging versus those without imaging**. Table S2.** Antiretroviral drug regimens received by mothers with HIV during pregnancy**. Table S3.** Adjusted mean differences in cortical thickness according to HIV exposure restricted to one site**. Table S4.** Adjusted mean differences in cortical thickness according to HIV exposure restricted to HEU children born to mothers on the same first-line ART regimen**. Table S5.** Comparison of cognitive, language and motor development between HEU and HU children**. Table S6.** Correlations between cortical thickness and language development stratified by HIV exposure**. Table S7.** Structural equation model

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Authors' contributions

CJW managed the neuroimaging data collection and was responsible for statistical analysis and drafting of the manuscript. KAD was responsible for the neuroimaging and developmental assessments and, with SY and DMG, assisted CJW with conception, supervision, and manuscript revisions. SS, AR, and JPF assisted with data collection, processing, and quality control. SJ and KLN provided imaging and visualisation advice, and along with AMR, provided input into the analysis. AMR performed data verification. HJZ is the principal investigator of the DCHS; DJS is the lead of the psychosocial arm of the parent study in which the neuroimaging is nested; both revised the manuscript critically for intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

The de-identified data that support the findings of this study are available from the corresponding author upon reasonable request as per DCHS cohort guidelines.

Declarations

Ethics approval and consent to participate

The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009, 525/2012 and 044/2017), the Western Cape Department of Provincial Health Research

Committee, and the London School of Hygiene & Tropical Medicine Observational/Interventions Research Ethics committee (11903). Written informed consent was obtained at enrolment into the DCHS, and mothers are reconsented annually. Additional written informed consent was obtained from the parent/guardian at the neuroimaging visit.

Consent for publication
Not applicable.

Competing interests
DJS has received research grants and/or consultancy honoraria from Discovery Vitality, Johnson & Johnson, Kanna, L'Oreal, Lundbeck, Orion, Sanofi, Servier, Takeda, and Vistagen. The other authors declare no competing interests.

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Publisher's Note

Part IV

General Discussion and Implications

11

Chapter 11: General Discussion

General Discussion

Summary

This chapter synthesises the results from chapters 4-10, interpreting them in the context of the existing literature to present the main thesis conclusions. Key strengths and limitations of the thesis are considered.

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11.1 Summary of research findings

Over one million children are born each year to mothers living with HIV. This thesis comprehensively assessed the early neurodevelopmental outcomes of children who are HIV-exposed and remain uninfected, and investigated the underlying brain structural pathways between HIV exposure and patterns of neurodevelopmental risk.

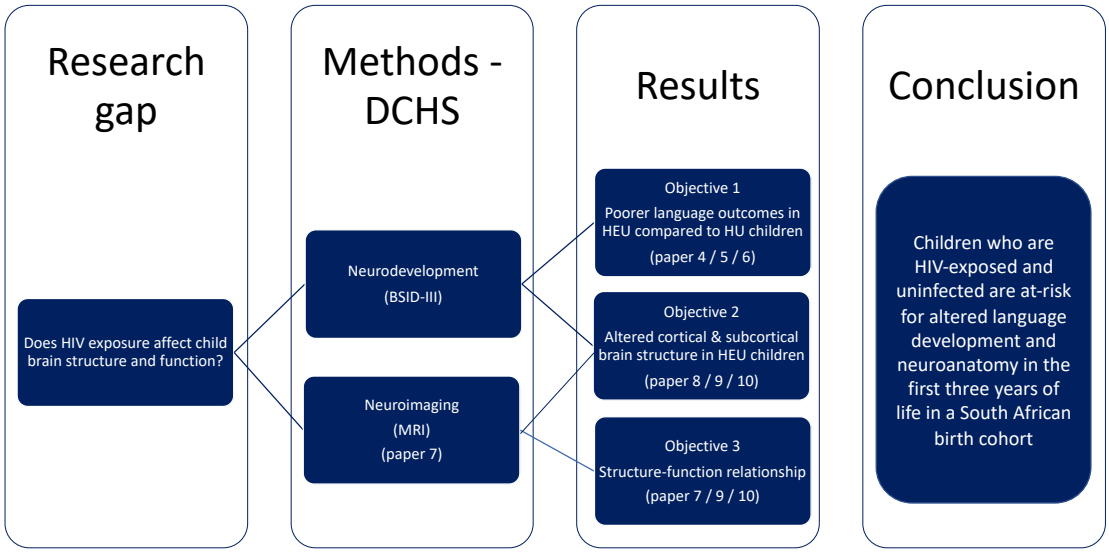

Figure 11.1: Summary of key research outcomes by objective

11.1.1 Aim

The aim of this thesis was to examine the effects of HIV exposure on the neurodevelopment and neuroanatomy of uninfected children born to mothers living with HIV in the ART era. The DCHS population-based birth cohort provided a unique opportunity to address this aim with comparative controls in a high HIV prevalence setting. Three objectives were met through the prospective study, while the systematic review and meta-analysis additionally contributed to objective 1 and the secondary objectives. (Figure 11.1l; Table 11.1)

11.1.2 Objective 1

To compare neurodevelopment (cognition, language, motor function) between children who are HEU and HU in infancy (6 months) and early childhood (2-3 years) [Chapters 4, 5, 6]

Overall, this thesis found that children who were HEU had poorer language development in early childhood compared to HU children. While no differences were detected at 6 months, by age 2-3 years children who were HEU demonstrated worse receptive and expressive language outcomes compared to HU children in the DCHS, with increased risk of language delay. The observed expressive language impairment was consistent with the results of the

large meta-analysis of eight studies, which additionally found a subtle difference in gross motor function, with lower scores in HEU versus HU children. There was no evidence of a difference in cognitive function between HEU and HU children in the prospective study or the meta-analysis.

11.1.3 Objective 2

To investigate group differences in brain structure in children who are HEU compared to HU using magnetic resonance imaging in early infancy (2-6 weeks) and early childhood (2-3 years) [Chapters 8, 9, 10]

The study demonstrated that neuroimaging young children in SSA without sedation or anaesthesia is feasible. Using high resolution neuroimaging, children who were HEU were found to have small but significant differences in brain structure at 2-6 weeks and at 2-3 years of age compared to HU children (Figure 11.2). On examination of subcortical structures, HEU children had lower volumes of basal ganglia nuclei at both age time-points compared to HU children (caudate at 2-4 weeks; putamen at 2-3 years), as well as reduced total grey matter in infancy and subcortical grey matter at 2-3 years; smaller hippocampal volume was additionally found at 2-3 years. Investigation of cortical structure found that HEU children had a trend for greater cortical thickness across the prefrontal cortex compared to HU children, with significantly thicker cortex in the medial orbitofrontal regions. No difference in cortical surface area was found in the DCHS cohort.

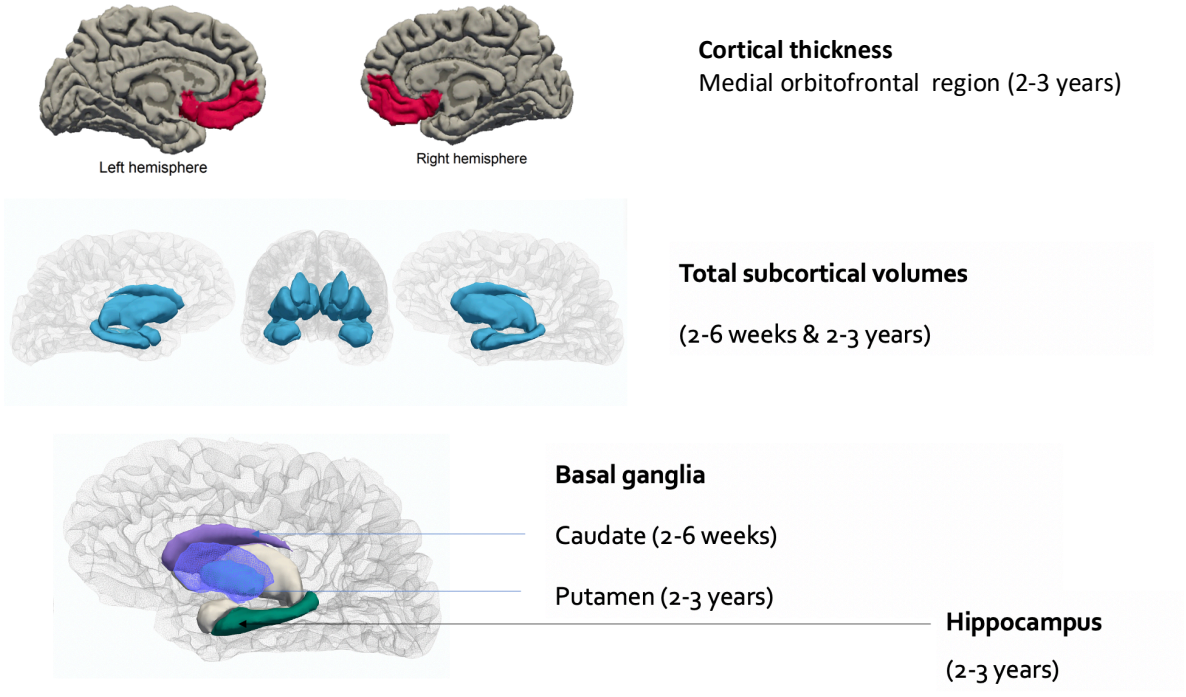

Figure 11.2: Illustrative summary of brain structure findings (Objective 2)

11.1.4 Objective 3

To determine whether there is an association between structural neuroimaging findings and neurodevelopmental performance in children who are HEU [Chapters 7, 9, 10]

Across the cohort, cortical structure in regions of the prefrontal cortex associated with neurocognitive scores at 2-3 years suggesting a brain structure-function relationship (Figure 11.3). Cortical thickness in the medial orbitofrontal regions mediated the association between HIV exposure and poorer language outcomes, explaining ~35% of language differences between HEU and HU children. Basal ganglia nuclei (putamen) and hippocampus volumes positively correlated with language scores at 2-3 years, suggesting that frontostriatal networks may be important pathways affected by HIV exposure that underlie language development.

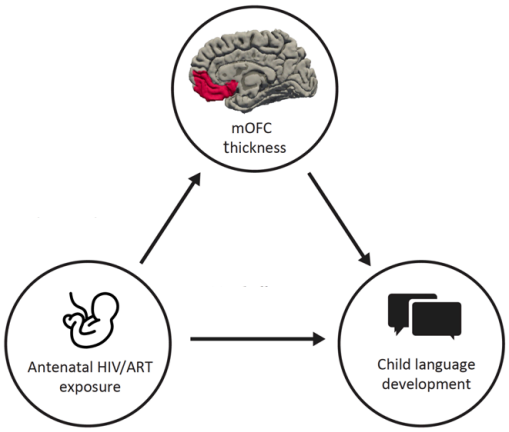

Figure 11.3: Illustration of mediation findings (Objective 3)

11.1.5 Secondary objectives

Secondary objectives include examining the associations between maternal severity of disease (as measured by CD4 and viral load) and ART with child neurodevelopment [Chapters 4, 6, 8, 9]

Lower maternal CD4 cell count in pregnancy was found to be associated with worse language outcomes as well as smaller grey matter volumes in infancy and at 2-3 years, while detectable maternal viral load was associated with smaller subcortical brain volumes at 2-3 years of age in a dose-response pattern. This suggests that maternal HIV disease severity and/or an activated inflammatory environment *in utero*, may be one mechanism by which HIV exposure affects child neurodevelopmental outcomes. The study found no evidence that maternal ART regimen or timing of ART exposure in pregnancy were associated with language or neuroanatomy. However, the majority of mothers in this study were on efavirenz-based regimens, limiting the investigation of specific ARVs in these relationships.

Table 11.1: Summary of key findings from the DCHS: Neurodevelopment and Neuroimaging

Outcome	Measurement tool	Timepoint	Participant *N*	Findings
Neurodevelopment (cognitive, language, motor)	Bayley Scales of Infant & Toddler Development (3rd edition; BSID-III)	6 months	260 61 HEU/199 HU	No group differences.
		2-3 years	732 168 HEU/564 HU	HEU children had worse receptive and expressive language, and greater delay versus HU children. Additional analyses showed continued poorer expressive language at 3.5 years in HEU children.
Subcortical brain structure	MRI	2-6 weeks	146 40 HEU/106 HU	HEU children had smaller basal ganglia nuclei (caudate) volumes (-5.4%) and total grey matter (-2.1%) compared to HU children.
	MRI	2-3 years	162 70 HEU/92 HU	HEU children had reduced subcortical grey matter (-2.9%) and smaller basal ganglia nuclei (putamen) (-4.7%) volumes and hippocampus (-3.4%) volumes than HU children. Volumes of these structures at 2-3 years positively correlated with language scores.
Cortical brain structure	MRI	2-3 years	162 70 HEU/92 HU	HEU children had greater cortical thickness in the medial orbitofrontal (mOFC) regions. Prefrontal cortical structure associated with neurocognitive outcomes. Cortical thickness in the mOFC region mediated the effect of HIV exposure on language.

Sub-analyses	Outcome tool	Timepoint	Participant *N*	Findings
Maternal ART regimen	MRI	2-6 weeks	Subgroups	No associations found.
Maternal CD4 in pregnancy	MRI BSID-III	2-6 weeks 2-3 years		Low maternal CD4 (<350 cells/µl) in pregnancy was associated with smaller grey matter volumes at 2-6 weeks (total) and 2-3 years (putamen). Similarly, lower CD4 associated with poorer language outcomes (raw scores and delay) at 2-3 years.
Maternal viral load in pregnancy	MRI	2-3 years		Maternal viraemia in pregnancy was associated with decreased subcortical grey matter volumes (putamen). A dose-response pattern was evident for subcortical, putamen and hippocampus volumes.

11.2 Comparison with the literature

11.2.1 Language development at risk in children who are HEU

This study found that, on average, children who were HEU had poorer language development and higher odds of developmental delay in receptive and expressive language compared to HU children at 2 years (Chapter 4). These results have been replicated more recently in large

studies in Botswana demonstrating adverse expressive language outcomes in HEU children at 2 years,[1] in Uganda where receptive language was impaired in HEU children aged 3 years,[2] and in the Zimbabwean Sanitation, Hygiene, Infant Nutrition Efficacy (SHINE) trial where HEU children had lower language scores than HU children at 2 years.[3] The meta-analysis of eight high quality studies across Africa and the USA (Chapter 6), similarly found worse expressive language (-0.17 (95% CI -0.27 to -0.07) in HEU versus HU children, with non-significant effects on receptive language (-0.10 (95% CI -0.23 to 0.03). In summary, the data indicate that children who are HEU are at risk for impaired neurodevelopment in language domains compared to HU children in the first years of life.

Language delay was detectable from age two years, and differences were not evident under one year (Chapter 4 and 6). Language function, which may be defined as 'an individual's ability to understand spoken language and to express themselves',[4] is relatively crude under one year and therefore subtle differences may not be detectable yet. While expressive language function is measured less often at older ages,[5] in a follow up analysis, we found that poorer expressive language in HEU children continues to be seen at 3.5 years in the DCHS with a similar effect size to two years (effect size: -0.23 [95% CI -0.45, -0.01] (Appendix XI). A prior US study identified language problems into adolescence, suggesting impairments may persist.[6] While the effect sizes for language differences are small, when applied to the growing population of HEU children this has potentially important implications. This thesis also reported the proportion of children with clinically significant language delay, with double the odds of developmental delay in HEU children compared to HU children (Chapter 4).

This is particularly concerning given early spoken language is predictive of school performance and associated with academic achievement at later ages.[7-9] Language underpins many aspects of learning and relationships with others, therefore language impairment can adversely impact academic, social, well-being and mental health outcomes.[10,11] Studies have shown associations between poorer early childhood speech and language with difficulties in school functioning, notably reading and mathematics, and higher rates of anxiety disorders and behavioural difficulties, compared to the general population.[12,13,14] Developmental language disorders may persist through adolescence into adulthood,[10,15] and children with language impairment may experience more disadvantaged socioeconomic circumstances later in life and limitations in psychosocial domains such as depression and employment.[16] affecting health-related quality of life trajectories through adolescence.[17] Conversely, higher language scores are associated with better health-related quality of life, notably in school and social domains.[18] Long-term follow up is important to understand the impact of these small

differences detected early in life in the DCHS, particularly since the children are young and language development is ongoing.

Despite substantial changes in contextual factors, including the introduction of ART and corresponding improved maternal health, the observed reductions in language functioning in HEU children are comparable to prior studies pre-ART in Asia and Africa.[19-21] This suggests that the mechanisms may be more biological, driven by HIV-specific pathways, which we list in the framework (Chapter 5) including: (i) direct exposure to HIV virions and viral proteins; (ii) maternal immune activation and inflammation; and (iii) fetal and infant immune activation and inflammation. Interestingly, impaired language function has often been reported in children living with HIV,[22] with language expression affected to a greater degree than comprehension.[21]

Reassuringly, there were no significant differences in cognitive or motor function between HEU and HU children at 6 or 24 months in the DCHS (Chapter 4). Previously, difficulties in both storage and processing in verbally-based tasks have been noted in HEU children, with the suggestion this may be due to greater demands and complexity in language skills compared to other tasks.[23] These results fit with literature showing preserved cognitive function in HEU children with increasing age.[5,24] However, the meta-analysis identified mildly reduced scores in gross motor function in HEU compared to HU children at two years (Chapter 6). The small effect size may have required greater power to detect than the DCHS afforded, and indicates the importance of large sample sizes and follow up.

11.2.2 Altered brain structure in children who are HEU

11.2.2.1 Grey matter volumes

The results of this thesis provide evidence for small neuroanatomical differences between HEU and HU children early in life with reduced grey matter volumes evident in early infancy and at 2-3 years of age. Subcortical grey matter was particularly affected, notably the basal ganglia nuclei at both age time-points (caudate in neonates, and putamen at 2-3 years), and the hippocampus at 2-3 years. There are very few neuroimaging studies of HEU children examining brain structure. One other South African cohort study of neonates has reported on subcortical volumes. This also found smaller basal ganglia nuclei volumes in HEU compared to HU children supporting the results, showing reduced putamen volumes overall, and smaller caudate volumes in HEU children born to mothers who initiated ART during pregnancy.[25]

The brain undergoes extensive growth in the early years,[26] with brain volume doubling in the first year, and reaching 80% of adult size by age two years.[27] Grey and white matter have different trajectories and there is particularly rapid development of grey matter volume in year one, increasing by 108-149% from birth.[27] Growth rates of subcortical grey matter structures are similar to cortical grey matter, and the caudate and putamen grow around 105% in the first year and 15% in the second, although trajectories vary.[26] Interestingly, the thesis results indicate that subcortical volumetric differences, notably in the basal ganglia, are detectable in infancy and remain evident through early childhood despite extensive volumetric growth during this period. Overall, the findings of smaller volumes in grey matter and subcortical structures suggest altered or delayed grey matter maturation trajectories in HEU children. Separately, the hippocampus regional differences were only detected at 2-3 years. While neurogenesis is largely complete at birth, the hippocampus is an exception and neurogenesis as well as neuroplasticity continue in this region, meaning it is particularly vulnerable to postnatal stressors and differences may manifest later. As an example, chronic stress has been associated with decreased grey matter volume in the hippocampus.[28] Therefore, a different mechanism may explain the hippocampus differences between HEU and HU children; or alternatively, varying regional trajectories of development may underlie the timing of detection.

The long-term clinical significance of these early differences remains to be determined. Research from another study of children living with HIV on ART found volumetric differences of a similar magnitude in the putamen, hippocampus, and global grey matter between children with HIV versus uninfected children (reductions of 4.6% right putamen, 3.9% left hippocampus and 2.5% global white and gray matter).[29] Separately, in a study of very preterm children (<30 weeks' gestational age) compared with term-born,[30] the putamen was found to be -6.2% smaller in very preterm children. In both groups, basal ganglia volumes were positively associated with motor, intelligence quotient and academic outcomes at age 7 years.[30]

11.2.2.2 Cortical thickness

To my knowledge, no other studies have investigated the effect of HIV exposure on cortical metrics. Cortical thickness development follows a U-shaped trajectory, increasing by around 35% initially, driven by dendritic arborization, axonal and glial growth, and synaptogenesis,[31,32] and peaking at around 1-2 years with some regional heterogeneity. This is followed by a decline which is both regressive (due to synaptic pruning) and progressive (due to myelination thinning).[33,34] The finding of thicker cortex at 2-3 years in HEU compared to

HU children may therefore represent a delayed or altered trajectory of development. Given there are minimal regional differences in gene expression during infancy,[35] this suggests that the observed altered patterns of cortical thickness growth are likely to reflect post-transcriptional processes due to environmental exposure, in this case HIV. One alternative explanation is that recent data suggest certain forms of stress in early life can cause 'accelerated maturation' as an adaptive top-down process (developmental support hypothesis) with subsequent reduced plasticity later in life.[36] Depending on the precise peak of the frontal regions, greater cortical thickness may also represent a faster maturation trajectory, and it will be important to determine this through subsequent neuroimaging at older ages.

While there are no other studies of cortical thickness in HEU children, the findings fit with other MRI data examining a variety of modalities indicating neuroanatomical and metabolite differences between HEU and HU children. Appendix III lists the existing neuroimaging studies of HEU children. Five others have focused on diffusion tensor imaging (DTI; one also reporting volumetric analyses), four on magnetic resonance spectroscopy, while only two papers have focused on structural scans. Overall, differences in white matter microstructure across ages have been described in neonates,[37] at 7 years,[38] and at 10-12 years,[39] along with neurometabolite alterations in HEU compared to HU children at 2-3 years[40] and 9 years.[41] Interestingly, some frontostriatal tracts and those connecting the hippocampus have been identified, and basal ganglia metabolite differences have also been reported. In terms of structural outcomes, Tardieu *et al,* 2005 paper included results following expert clinical evaluation (i.e. non-volumetric focus) of scans and reported over 50% had abnormalities.[42] However, this study had major limitations as there was no comparison group (clinical cohort), brain images were obtained retrospectively without standardized acquisition parameters, children were selected based on neurological symptoms, and were predominantly exposed to zidovudine. Similarly, the DTI paper by Jahanshad *et al,* 2015 includes a comparison of brain structure, however, this was conducted in older children, approximately half were not exposed to ART, and there was no quantitative differentiation of subcortical volumes between exposed and unexposed groups.[43]

Although there is heterogeneity, prior research of children living with HIV has shown similar associations, and direction of effect, between HIV infection and brain volumes. Studies in children,[29] adolescents,[44] and adults,[45] with HIV have reported smaller grey matter volumes (global and subcortical) despite early initiation of ART, as well as cortical thickness alterations;[46] and basal ganglia calcification.[47] One paediatric study found reduced volumes of the right putamen, left hippocampus, and global grey and white matter and thicker cortex.[29] This pattern of structural changes in subcortical grey matter as well as frontal regions matches

a signature reported in adults living with HIV who are stable on ART with viral suppression.[48] Interestingly, another study examined various structural metrics and found cortical thickness was the most sensitive for structural abnormalities related to HIV across ages; language fluency, attention and motor scores were correlated with mean cortical thickness.[49] Overall, these data suggest that there may be similarities across neurobiological pathways for the impact of HIV-related neural damage to the developing brain in the context of exposure and infection; or the downstream effects of altered immune function in pregnancy may have a similar impact.

11.2.3 The brain structure-function relationship

The results suggest that regional grey matter plays a key role in neurocognitive and language development (Chapter 7). This adds to the evidence base that early brain growth forms the structural underpinnings of child development and function.[26] The research underscores the importance of the *in utero* period, the time of most rapid brain development,[50] and that alterations to typical brain trajectories in this period have the potential to impact neural networks and later neurobehaviour.[51,52]

Language requires complex pathways and therefore may be more affected by HIV exposure than other functions. This study found that both prefrontal cortical thickness and subcortical volumes were associated with language scores in children. In particular, HIV exposure was associated with cortical thickness in the medial orbitofrontal region of the prefrontal cortex, which was found to mediate 35% of the effect of HIV exposure on language function, as well as basal ganglia nuclei and hippocampus volumes which correlated with language outcomes. These findings fit with a recent meta-analysis that identified a pattern of neuroanatomical abnormalities in the basal ganglia, specifically in the neostriatum (caudate nucleus and putamen), associated with developmental language disorders.[53] The authors hypothesised the basal ganglia may influence language through a role in procedural memory.[54] The frontal cortex showed the second most structural anomalies associated with developmental language disorders.[53] Overall, this neuroanatomical signature aligns with our findings.

Prefrontal regions are connected to basal ganglia structures (putamen and caudate nuclei),[55] via frontostriatal circuits.[56] The frontostriatal networks are established in pregnancy and are well-described as being foundational for learning and memory.[55] It is possible frontostriatal connectivity may be affected by HIV exposure, driving the poorer language outcomes. Of note, frontostriatal circuits have also been implicated in pathology of HIV infection. Separate connections link the prefrontal cortex to the hippocampus,[57] and these may also be affected by volumetric alterations in the component structures, affecting function. This is consistent with

growing evidence that neurocognitive function relies on distributed neural networks with dynamic interactions between brain areas, including subcortical and cortical structures, that work in parallel circuits.[58] Notably the orbitofrontal cortex is part of the limbic-emotion circuit responsible for decision making and rewards, together with the basal ganglia,[59] indicating a need to follow up with these children to monitor later language and behaviour patterns.

Overall, the results suggest that HIV exposure may affect regional brain maturation at a critical time of CNS development (Figure 11.4), influencing child neurobehavioral trajectories. Altered neuroanatomy may act as an intermediary phenotype mediating language outcomes,[60] and the degree of alteration in cortical and subcortical morphometry, or the resulting effect on frontostriatal connectivity, may influence function.[61] Although there was no evidence for a difference in motor development in children in the DCHS, the meta-analysis identified subtle reduced gross motor scores in HEU compared to HU children. It is therefore worth noting that the frontostriatal system (notably including the orbitofrontal cortex, caudate and putamen) has been associated with specific motor behaviours.[62] It remains to be seen whether these changes are markers of risk for future neurocognitive and functional outcomes.

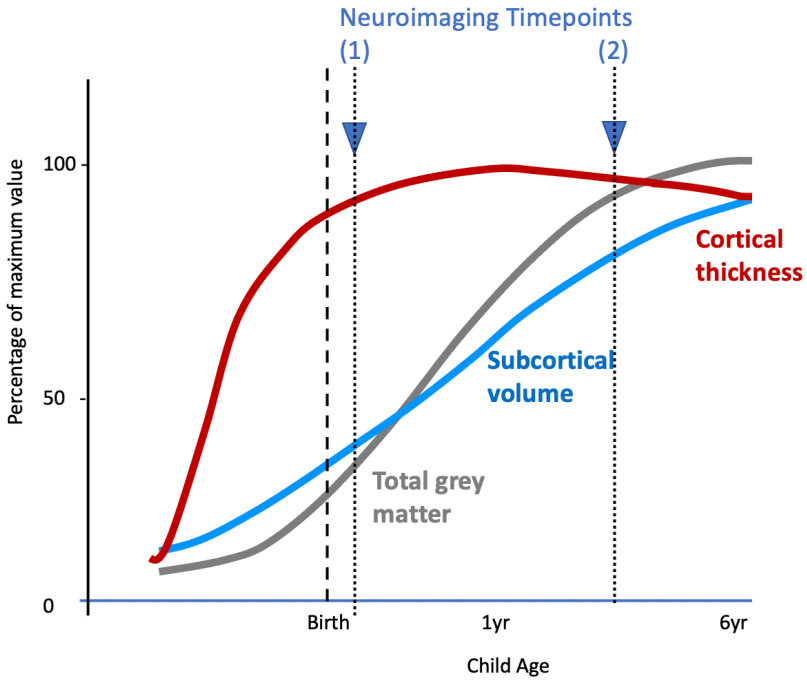

Figure 11.4: Mapping brain trajectories of key MRI metrics reported in this thesis using a child brain growth chart. This figure contextualises the study findings through illustrating normative child brain trajectories of total grey matter and subcortical volume and cortical thickness across the early years. The two neuroimaging timepoints are shown. Child brain growth chart adapted from Bethlehem *et al.* 2022. Nature.[50] Reprinted by permission as indicated in the Terms and Conditions of the license CC-BY-4.0.

11.2.4 The role of maternal HIV disease severity

The identified associations between lower maternal CD4 cell count and higher viral load in pregnancy with smaller brain volumes in children suggest that maternal HIV disease severity and corresponding immune activation may be important elements to consider. Maternal immune compromise may affect the highly orchestrated process of brain development (Chapters 4, 8 and 9). The identified dose-response associations between maternal CD4 cell count and viral load and grey matter volumes are indicative that some of the group effects may be driven by children born to mothers with more advanced HIV disease, suggesting an underlying biological mechanism, while also providing a direction for potential prevention strategies. The results are similar to a recent study which found a negative association between putamen volume and maternal viral load.[25]

The maternal immune system plays a crucial interactive role in brain development.[63] In early life the developing brain is particularly vulnerable to environmental exposures, as the complex processes of neurogenesis, neural migration, synaptogenesis, myelination, circuit formation and pruning progress. Therefore, it may be hypothesized that immune changes *in utero* will have the greatest effect. There is increasing recognition that exposure to maternal infections in pregnancy, even without congenital infection, can affect neurodevelopment. This includes TORCH infections (toxoplasmosis, other [syphilis, hepatitis B], rubella, cytomegalovirus, herpes simplex), and then Zika virus in recent years. Of note, antenatal exposure to infection is an established risk factor for neurodevelopmental disorders in children.[64,65] This aligns with the *developmental origins of the health and disease model* that describes how fetal adaptation and plasticity in response to antenatal exposures influence infant brain-behaviour outcomes and the risk of neuropsychological disorders.[60]

Acute and chronic infections in pregnancy have been found to induce fetal changes without direct infection of the fetus through inflammation and/or maternal immune activation.[66] Maternal immune activation describes the 'systemic increase in inflammatory markers during pregnancy, traditionally in response to infectious exposures, associated with poor outcomes,'[63] and this has been reported in pregnant women living with HIV.[67] Infection-induced maternal immune activation and inflammation mean the fetus is exposed to proinflammatory factors which can lead to infant immune dysregulation. Overall, this may be hypothesised to affect developing neural circuits, such as frontostriatal connections, during critical windows of development. In support of this hypothesis, prior DCHS research has shown altered maternal and infant immune profiles and associations with neurodevelopmental outcomes.[68] Further, the research in Appendix XII suggested a role for neuroinflammation,

reporting a pattern of neuroinflammation in HEU children at two years using magnetic resonance spectroscopy. In animal models, offspring exposed to maternal immune activation display changes to neuronal dendritic structure (branching and diameter) in the prefrontal cortex.[69] In other infections, persistent immune activation has been associated with developmental abnormalities in children, potentially mediated by microglial (immune brain cells) priming.[70] Microglia-mediated neuroinflammation in the basal ganglia, and specifically the caudate nucleus, has also been implicated in adverse paediatric development and disorders.[71,72] Overall, deviations from normal immune function, including neuroinflammation, may have a pathophysiological influence on neurodevelopment.

A range of immune activation has been documented in women with HIV and a number of factors likely contribute to this including timing of HIV acquisition and ART, the environment, and genetic risks that remain to be determined.[67] Detectable viral load and low CD4 are also reflective of increased exposure to HIV-related virions and proteins that could affect fetal brain structure directly, as well as via corresponding immune activation changes, or through the relationship with other coinfections.

11.2.5 The role of maternal ART

There was no evidence of a relationship between maternal ART regimen or timing and child brain development in the DCHS. However, the majority of mothers in the DCHS were on efavirenz-based regimens and there may not have been sufficient power to detect ARV-related differences. Efavirenz has been linked to neurological adverse effects in people living with HIV,[73] and to worse child language outcomes in HEU children.[74] Therefore, further work is needed to determine whether neurotoxicity may be a contributing factor. Another study found a positive association between caudate volume and longer duration of maternal ART exposure,[25] the authors concluded that this may be reflective of earlier ART initiation (prior to conception) offering protection of the maternal immune system and child outcomes. There was also no consistent signal between maternal ART regimen and neurodevelopment from the systematic review (Chapter 6), however, data were scarce and the introduction of new ART regimens requires further study. While the benefits of maternal ART far outweigh any risks, understanding the most optimal ART regimen in pregnancy for child outcomes is a future research priority. Dolutegravir is now the recommended first-line regimen and new studies are required to assess integrase inhibitors (see further work in Chapter 12).

11.2.6 Other socioeconomic, environmental, and biological factors

Neurodevelopment is a complex process affected by multiple socioeconomic, environmental, and biological factors,[75] as detailed in the Conceptual Framework (Chapter 4). HIV exposure exists within a range of factors including socioeconomic and family variables, and children born into HIV-affected households face multiple risks that may influence their neurodevelopmental outcomes. Potential confounders were considered in each paper and the findings held after adjusting for relevant covariates. Nevertheless the cumulative layers of risk are difficult to measure and there is a constellation of interacting genetic and environmental factors that impact this complex physiology. Particular consideration was given to biological, social and structural variables in the supplemental paper in Appendix XI where birthweight, child sex, maternal education, maternal ART duration and HIV viral load during pregnancy were found to influence HEU child language outcomes at 3.5 years. Further, on examining neuroanatomy in Chapter 10, brain structural alterations explained ~35% of the effect of HIV exposure on language outcomes, indicating the existence of other pathways. Understanding the contributing factors and mechanisms underlying the identified developmental delays and structural brain alterations require further investigation. Details of future research into contextual factors are listed in Chapter 12.

11.3 Study Strengths

This study has many strengths. Brain structure and function were investigated in a large, deeply phenotyped sample of HEU children and a comparative HU group from the same environment using comprehensive neuroimaging and validated developmental tools. Data were collected prospectively, limiting bias.

11.3.1 Well-characterised cohort

The DCHS is unique as it collects data on several biological, psychosocial, and environmental variables. This allowed for a rich and extensive information base and for the environmental context to be considered. As a result, many more socioeconomic and HIV-related variables could be accounted for than prior global imaging studies on brain outcomes of HEU children.[39,76,77]

11.3.2 Control group

Potential bias and confounding were mitigated through the inclusion of a control group recruited from the same community. Sociodemographic characteristics (including maternal education, employment, and relationship status) were comparable between HEU and HU groups and between the full cohort and the neuroimaging sub-group. Potential confounders

were carefully considered in sensitivity analyses. Additionally, for the neuroimaging sub-group, children who were high-risk for neurodevelopmental delay were excluded to reduce confounding (see Chapter 3 Methods). This may have led to a reduction in effect size given HIV and ART exposure have been linked to adverse birth outcomes,[78] however, differences were still observed suggesting the effects were robust.

11.3.3 Validated early-life assessment

Neuroimaging and neurodevelopment were assessed in early life when outcomes have relatively less confounding from postnatal factors. Inherent challenges to MRI in young children mean there is a lack of studies in younger age groups when the most rapid development occurs. This study began to address the dearth of literature from SSA on brain development in children who are HEU in early life.[50]

11.3.4 Multiple timepoints

The study assessed brain structure and function at multiple timepoints, allowing the results to be interpreted in the context of brain growth. Temporal associations give strength to the conclusions. Overall, the findings represent the first prospective African cohort study to examine longitudinal neuroanatomy of HEU children from infancy and demonstrate neuroanatomical changes are present soon after birth and remain evident at 2-3 years. These changes correlate with functional outcomes widely described to be impacted in HEU children.

11.3.5 Study representativeness

The DCHS cohort is from a low socioeconomic population with a high prevalence of psychosocial risk factors, representative of many pregnant women living with HIV worldwide, making the results potentially generalisable.[79] In the catchment area of the Drakenstein sub-district where the majority of births occurred at Paarl Hospital, the study enrolled approximately 10% of births.[3] This represents 0.03% of all births in South Africa during the study recruitment period.[4] In the cohort, 48% of women received national social assistance and 39% completed secondary education or higher,[80] which is comparable to the rest of South Africa where 46% households received ≥1 grant and 46% of people had ≥grade 12 qualification in 2019.[81] In terms of HIV, South Africa has the highest numbers of children who are HEU worldwide, and over 21% of children are born to mothers living with HIV each year.[82] Similarly, antenatal HIV prevalence in the DCHS is 21% across the cohort,[83] and maternal CD4 cell counts in pregnancy parallel a national study of women with HIV.[84] Coverage of pregnant women who receive ARVs for PMTCT has been high since 2013, with minimal change subsequently,[85] indicating these data continue to be relevant.

11.4 Study limitations

The thesis has several limitations that particularly relate to the nature of observational study and are documented below. Additionally, each results paper includes discussion of the specific limitations (Chapters 4, 5, 7, 8, 9, 10) and the systematic review and meta-analysis details limitations relevant to that paper (Chapter 6).

11.4.1 Selection of participants

This study was nested within the DCHS population-based birth cohort study. While loss to follow up was minimal, around 10% in the first two years, the possibility of selection bias remains. Every effort was made to improve cohort retention and reasons for loss to follow up were reported (Chapter 4). The neuroimaging study included a sub-group of the full cohort due to financial and logistical reasons (scan availability and location). To assess for selection bias, sociodemographic comparisons between the neuroimaging sub-group and full cohort were conducted for each analysis. These indicated that the sub-group was representative (Chapters 7, 8, 9, 10). Due to internal migration and scanning logistics, it was not possible for all mother-infant pairs who had neonatal imaging to return for the 2-3 year old scans. Substantial effort was made to include these mothers, and additional children were recruited from the DCHS to reach the appropriate sample size. This ensured sufficient power to address objective 2, however, it precluded true longitudinal trajectory analyses. There were also challenges inherent to the nature of neuroimaging young children during natural sleep. Children who did not sleep or moved were excluded as this affected image quality. We found no associations between scan success and neurodevelopment, but this reduced the sample size (Chapter 7). Overall, the sample was still large for a neuroimaging study, fulfilled the sample size calculations, and the effect sizes are in keeping with those reported in other studies,[86-88] however, the study may have been underpowered to detect smaller differences. Similarly, the exploratory analyses of viral load and CD4 had lower numbers as maternal viral load data were not routinely collected in the earlier years. Future studies are needed to replicate findings and address potential interactions between CD4, viral load and ART.

11.4.2 Changing HIV/ART guidelines

The evolving nature of the HIV treatment and prevention landscape places limitations on the generalisability of the results. Although no associations were seen between ART exposure and brain outcomes in this study, it is difficult to disentangle the effects of HIV and ART and more work is needed (see section 11.2.5). Studies of children who are born to mothers without HIV who are taking pre-exposure prophylaxis (PrEP) may help to better understand the effects, if any, of ART on child neurodevelopment. Since the study began, the HIV

guidelines have changed and new WHO guidelines recommend dolutegravir-based ART as first-line for women of child-bearing age.[89] Dolutegravir has increased efficacy at reducing HIV viral load than NNRTI-based ART,[90] with implications that this may lead to immune protection and better child outcomes. However, previous concerns were raised about the use of dolutegravir at conception and its potential link to CNS development, particularly neural tube defects.[91,92] Although these concerns were later refuted,[93-95] neural tube defects are related to abnormalities in embryogenesis. Therefore, it is possible that factors affecting this process could also influence other aspects of CNS development including neurogenesis, synaptogenesis, and myelination. Understanding the outcomes of HEU children exposed to integrase strand transfer inhibitors (INSTIs) now becomes a key priority. Although pre-dolutegravir, the thesis results provide valuable comparative data with regimens that have been current for the best part of a decade and to which millions of children have been exposed.

11.4.3 Neurodevelopmental tools

The BSID assessment is recognised to be the most widely used measure of neurodevelopment in this age group worldwide. The third edition has been validated in a South African setting.[96] However, there remain concerns over the standardisation in SSA, and that the norms may over-estimate development, resulting in children obtaining higher than expected scores and leading to underestimation of delay.[96-98] In Chapter 4, both continuous and categorical scores are reported alongside raw and scaled scores, with similar results. However, underestimation of delay may have contributed to the lack of association with cognitive and motor outcomes. There are also limitations to the assessment including human errors with scoring and administration. These limitations were addressed by instituting quality control measures. Given the concerns around the appropriateness of US normative data, the results were compared with a matched HU group from the same cohort and setting, rather than against the norms. Finally, other sensory factors may have influenced language delay. However, we noted any hearing or other impairment affecting assessments and formal hearing assessments (otoacoustic emissions and tympanometry) were also conducted in the DCHS.

11.4.4 Confounding and residual bias

There is a risk of confounding from socioenvironmental factors in neurodevelopmental assessments. Potential bias was mitigated through (i) the use of a control group recruited from the same environment; (ii) comparing sociodemographic characteristics of HEU and HU groups; and (iii) careful consideration and interrogation of potential confounders and

sensitivity analyses. However, there is the possibility of residual bias from unmeasured factors.

Notably, when examining the role of environmental factors, the HU control group had higher alcohol exposure compared to the HEU group. Sensitivity analyses were specifically performed across the neurodevelopment and neuroimaging analyses to ensure the results were not confounded by maternal alcohol use. However, associations have been identified between prenatal alcohol exposure and brain outcomes, including reduced brain volumes in subcortical regions and alterations to other cortical morphological measures. [99-102] Therefore, this may have decreased the measured effect size of HIV exposure. The DCHS also had a low rate of exclusive breastfeeding which means it may not have been possible to adequately control for, or understand, the potential protective effects of breastfeeding.[103,104] Findings need to be replicated in other cohorts with less alcohol exposure and higher breastfeeding rates. Further research exploring underlying causal pathways and accounting for the complex interactions between factors that impact brain maturation, including the role of family and parenting, is also needed (Chapter 12).[105]

In considering the role of child biological sex, prior literature has established that this may influence neurodevelopmental outcomes.[106-109] The analyses in the neuroimaging chapters were not stratified by sex due to power constraints of the sample size. However, child sex was included as a covariate in all multivariable analyses. Intracranial volume was also included as a covariate in volumetric and cortical surface area analyses to account for overall brain size differences.[110] Further research is needed to understand sex differences in neurodevelopmental trajectories, including the greater risk of cognitive impairment identified in male CHEU (Appendix XI) and any sex-specific mechanisms.

11.4.5 Causality

The observational nature of the study means that it was not possible to establish causality. HIV exposure represents a range of factors that may influence neurodevelopment,[111] including altered immune function, direct effects of HIV, ART exposure, and wider psychosocial and economic factors associated with being born into a family living with HIV; future research needs to attempt to tease these apart. In particular, there are difficulties separating out the effects of HIV and ART. The nature of this thesis timeline also meant that there were constraints in duration of follow up. Therefore, it was not possible to extrapolate the long term clinical impact of the findings. Further prospective studies are needed to investigate the clinical significance and ongoing effects of HIV exposure at older ages.

Finally, cross-sectional analyses were performed as only 33% of children had high-quality scans at both the neonatal and 2-3 year timepoints, which reduced the power to conduct longitudinal analyses. However, longitudinal analyses are important, and research is ongoing at older ages (see Chapter 12 Future work) which will allow a comparison of trajectories.

11.5 Conclusions

The successful scale-up of ART during pregnancy has resulted in a substantial decline in infant HIV infections and most children born to women living with HIV remain uninfected. While we need to celebrate the reduction in vertical transmission, worldwide there are 16 million children who are HEU, the majority living in SSA, and their neurodevelopment is of substantial public health importance.[111] This thesis assessed the effect of HIV exposure on child brain structure and function utilising neuroimaging technology. The results found that HIV exposure is associated with an increased risk of language impairment in early life and altered structural brain development, including smaller regional brain volumes and thicker cortices. Associations between HIV exposure and structural brain development in regions that influence language processes may underlie functional delays. This has implications for later life given early language is associated with academic, social, and mental health outcomes and long-term follow up is needed. Children who are HEU continue to face a multiplicity of risk factors. The work provides a unique insight into the effect of HIV exposure on the developing brain during a critical window for potential intervention.

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12

Chapter 12: Clinical, Policy, and Research Implications

Clinical, policy, and research implications

Summary

This study followed children who are HEU across the first three years of life and found differences in neurodevelopment and neuroanatomy when compared to their HU peers. There appears to be a relationship between antenatal HIV exposure, early maturation of specific brain structures, and language outcomes, as well as an association with maternal immunosuppression. This final chapter reflects on the clinical and public health implications of the work as a whole and how the findings may inform prevention and intervention strategies to improve neurodevelopmental outcomes of children who are HEU.

Avenues for future research are considered including: defining longitudinal neurodevelopmental trajectories; conducting multimodal neuroimaging assessments; and understanding causal and mechanistic pathways. Consideration is also given to how the findings may be extrapolated to the wider developmental literature. A penultimate section discusses the importance of education and public engagement, and describes a community engagement project on the topic of neurodevelopment. The thesis ends with a final conclusion on the work.

Parts of the text in this chapter relating to clinical practice and policy are adapted from the same book chapter cited in the Introduction:

Book chapter: Wedderburn CJ, Yeung S, Donald KA. Neurodevelopment of children who are HIV-exposed uninfected. In: Recent Advances in the Neurological and Neurodevelopmental Impact of HIV. Editors: Amina Abubakar, Kirsty Donald, Jo Wilmshurst, Charles Newton. Mac Keith Press 2023.

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12.1 Implications for clinical practice and public health policy

The remarkable reduction in vertical transmission of HIV in recent years is a reason to celebrate.[1] Alongside this success, this thesis indicates that children with perinatal HIV exposure who are uninfected remain a vulnerable population. This body of work adds to our understanding of HIV exposure as a risk factor for poor neurodevelopment in the early years, affecting those children who remain HIV-free as well as those living with HIV. The research suggests that children who are HEU are at risk of early language impairment as well as structural brain differences.

These findings have public health implications, as language is important for many aspects of life including social interactions and educational progress. Early neurocognitive skills influence schooling, health, and social potential,[2,3] and even subtle neurodevelopmental impairments have the potential for adverse long-term outcomes, both at an individual and a societal level.[4] Notably, language skills are foundational for school readiness and predict later literacy, academic achievement, mental health, and social well-being.[4] Epidemiological studies suggest that language difficulties in young children may persist into adolescence.[5] Therefore, poorer early-life language development heralds concerns for school and academic attainment, with implications through adulthood. Correspondingly, low language abilities have been associated with literacy, reading and writing, participation,[6] and behavioural difficulties as well as limitations in psychosocial and school functioning,[7-9] and socioeconomic circumstances later in life.[10]

The Sustainable Development Goals have made early child development a priority on the global agenda, emphasising those who are vulnerable and at-risk. Given the growing population, any language impairment in children who are HEU has substantial public health relevance, particularly in countries where HEU children represent a high portion of annual births. Promoting optimal language development is now a public health priority. This research underscores the importance of prevention strategies and monitoring child language development to recognise and address any problems early, thereby preventing future academic, social, and mental health difficulties. Potential implications for clinical practice and public health policy from this research are discussed below (Table 12.1).

Table 12.1: Implications of study findings for public health and clinical practice, research, and community engagement

Public Health & Clinical Practice	
Primary prevention of HIV in women	Provide early integrated care - nurturing care framework
Optimise health and HIV care of mothers living with HIV; avoid immunocompromise	Targeted intervention strategies for children who are HEU
Monitor and screen neurodevelopment of HEU children	Identify and support those with developmental delay
Research	
Investigate causal and mechanistic pathways	Define longitudinal neurodevelopmental trajectories to understand how early findings map onto later outcomes
Conduct multimodal neuroimaging assessments to further delineate underlying changes	Extrapolate findings within the wider developmental literature
Community Engagement	
Understand community perspectives; education on maternal & child health during pregnancy and post-partum	Family-centric approach to reduce stigma; inform parents about early identification & simple interventions

12.1.1 Public health perspective and prevention strategies

12.1.1.1 Primary prevention of HIV in women

From a public health perspective, it is essential to continue to promote primary prevention of maternal HIV for optimal child outcomes, as well as maternal health. Focusing on preventing HIV acquisition, particularly in adolescents and young women, will prevent HIV exposure in children. Over time the transmission landscape has changed with a relative increase in postnatal compared to antenatal/perinatal transmission. Ensuring prevention efforts are maintained throughout the breastfeeeding period is important to limit postnatal HIV exposure.

12.1.1.2 Optimise health and HIV care of mothers living with HIV

The findings suggest directions for optimal HIV management strategies in mothers living with HIV to improve child HEU brain outcomes. The neonatal imaging results (Chapter 7) suggest neuroanatomical changes in HEU infants are identifiable soon after birth, prior to exposure to other postnatal factors, indicating a key role for the uterine environment. The association of maternal CD4 cell count with child language outcomes and grey matter volumes (Chapters 4, 8 and 9) and viral load (Chapter 9) indicate the importance of maintaining maternal immune health and reducing viral load in pregnancy. Preventing

immunocompromise of mothers in pregnancy (and pre-pregnancy) appears to be essential for optimal fetal neural development. This work also supports other studies indicating maternal disease severity is a risk factor for child neurodevelopment.[11,12] Strategies for ensuring effective HIV care and reducing immunocompromise include early HIV diagnosis and ART initiation, close viral load monitoring and maternal HIV management during pregnancy, optimising ART adherence (alongside investigating the best ART regimen in pregnancy for child outcomes), patient education, and supporting maternal psychological well-being.

12.1.1.3 Monitor and screen children who are HEU

The findings indicate that careful monitoring of neurodevelopment (Chapters 4-6), particularly language function, coupled with early identification of any impairment and intervention where needed, is important for children who are HEU. In many contexts, the growth and development of all children is monitored at various intervals over the first five years of life. Any screening systems (general or specific) should consider HIV exposure as a factor for developmental risk, and healthcare professionals conducting these assessments should pay particular attention to HEU children. Early identification of concerns or delays in language development is vital for management to be optimised; therefore, it is important for healthcare professionals to be aware of possible vulnerability to the speech and language development of this at-risk population. Additionally, as language deficits may only become evident around two years (Chapter 6), follow up to older ages is necessary.

Detailed neurodevelopmental follow up in countries where children who are HEU make up over 20% of the population is difficult. Various neurodevelopmental tools have been developed in LMIC settings to ensure validity in diverse contexts including the Malawi Developmental Assessment Tool (MDAT) and Kilifi development index, the neurodevelopment screener in Bangladesh, and more recently the Global Scales for Early Development (GSED). However, it remains challenging to integrate these into the health system given multiple factors including: (i) the time taken to conduct assessments; (ii) limited expertise coupled with training requirements; (iii) variable sensitivity to picking up impairment, particular to early language; (iv) different approaches to domain measurement and the need to adapt across contexts; and (v) capturing children not accessing the health system. Simplifying assessments and selecting screening tools that will be appropriate to enable follow up at a population level is critical.[13] Further, research on long-term follow up of children who are HEU is also important to understand if early deficits translate to later outcomes and to inform the need and cost effectiveness of detailed assessments.

12.1.2 Intervention strategies

12.1.2.1 Early integrated care for all children

Accumulating research shows that the accrual of risk factors puts children at greatest risk for not reaching their developmental potential. This has led to a paradigm shift away from siloed interventions to focus on optimising development through reducing multiple risk factors and enriching the environment to optimise outcomes.[14] While this research shows HIV exposure puts children at risk of poorer developmental outcomes, children born into HIV-affected households experience other risks as well. In multi-risk environments, a general holistic approach following the Nurturing Care Framework (https://nurturing-care.org/) may be taken, with assessment to detect problems, and implementation of supportive integrated interventions to ensure all children are able to reach their full potential. Nurturing Care covers several core areas including: good health, adequate nutrition, responsive caregiving, safety and security, and opportunities for early learning. Optimising these components is best achieved by taking a community-focused approach, through education, and encouraging positive family environments, nutrition, general health, vaccinations, and parent-child interaction. Measures such as early home visits,[15] stimulation programmes,[16] and interventions to optimise the home environment,[17] have shown positive results. While this thesis suggests that the brain is vulnerable to HIV exposure in early life, this also represents a period of opportunity. In this critical early period the brain has most potential for plasticity and is more responsive to positive stimulation.[14,18] Building on this, any management strategies need to be initiated early to have the greatest chance of preserving developmental potential.[19]

12.1.2.2 Targeted intervention strategies for children who are HEU

The identified associations between HIV exposure, language (and motor) outcomes, and the structural brain differences reported suggest that additional interventions may be needed to optimise the development of HEU children. This highlights the importance of identifying targeted interventions and conducting studies to assess scalable packages of care for early HEU child development as a priority. A randomised trial in rural Zimbabwe found infant and young child feeding in combination with water, sanitation, and hygiene (WASH) interventions improved motor, language, and behavioural scores in children who are HEU.[20] Separately, a systematic review of interventions identified a few small studies that suggested massage therapy gave improvements in developmental domains measured, whereas mixed outcomes were reported for caregiver training and cognitive therapy interventions; however, caution needs to be taken when interpreting these results given the sample sizes.[21] Some randomised controlled trials of early child development interventions involving training

caregivers in impoverished areas of Uganda have been conducted focusing on HIV and HEU children.[22] While an intervention for caregivers was found to improve child language,[23] a cluster-randomised trial found caregiver training improved caregiving quality but did not significantly impact neurodevelopment of children who are HEU compared to a health and nutrition training intervention in that context.[24] Overall, evidence is limited and further work is needed to determine scalable and effective targeted interventions combined with implementation research. In addition, it will be important to understand whether early imaging changes may be used to assess effectiveness of interventions.

12.1.2.3 Identify and support those with developmental delay

Any child suspected of having developmental delay should be formally assessed to ascertain the degree and profile of developmental impairment and potential contributing factors. Investigations should be tailored to both presentation and context, and the role of different factors that can influence neurodevelopment of a child who is HEU should be considered (see Chapter 5). Management will depend on availability of care in the specific context, and further information may be found in the book chapter in *Recent Advances in the Neurological and Neurodevelopmental Impact of HIV*.[25]

12.2 Future Research

Future research is needed to expand, understand, and replicate the findings in different contexts. Further studies examining the neurodevelopment of HEU children may consider the methodological suggestions identified in Chapter 6. Potential areas of work include characterising the impairment as children age; understanding risk and delineating the specific mechanisms; and extrapolating to the wider developmental field. Areas of ongoing research are described.

12.2.1 Defining longitudinal neurodevelopmental trajectories

Long-term follow up is needed to understand how both the structural and functional developmental differences in early life translate to later outcomes to inform monitoring and intervention strategies. Brain growth continues throughout one's lifespan.[26] The thesis results found neurodevelopmental and neuroanatomical differences between HEU and HU children across the first three years. Defining longitudinal trajectories of neurocognitive function and brain growth of children who are HEU is needed to ascertain whether the identified differences are transient, remain stable, or change with time. Understanding how early imaging and neurodevelopmental assessment outcomes map onto longer term HEU child

outcomes (e.g., school readiness and performance, and neurobehavioural disorders) may inform targeted interventions.

Few studies have longitudinally examined neurocognitive development of children who are HEU in LMICs.[27] While some studies have reported similar outcomes between HEU and HU children aged 4-11 years,[12,28-31] others found poorer school outcomes,[32] lower IQ, and delays in language and fine motor development in HEU children.[33] Notably, expressive language function is not often examined, however, a US study found perinatally HIV-exposed youth aged 9-16 years continue to be at risk for persistent language impairment.[34,35] Recent data from the DCHS at 3.5 years suggests the expressive language impairment in HEU children persists, with a similar effect size to the two year age point (Appendix XI). Longitudinal neuroimaging studies through childhood also remain limited and are notably lacking from Africa. A handful of studies have reported on HEU children older than five years using diffusion tensor imaging and magnetic resonance spectroscopy (see Appendix III). While one study examined whole brain structure at 10 years,[36] there are no studies quantitatively reporting on targeted analyses of grey matter, cortical, or subcortical structures.[26] Neuroimaging at older ages will provide novel data in the HEU population.

12.2.2 Conducting multimodal neuroimaging assessments

The study found that neuroimaging young children without sedation is feasible in a sub-Saharan African setting. The techniques developed in this research can be used in other studies to help with neuroimaging children. Further, the introduction of low-field MRI techniques, such as the 64mT Hyperfine Swoop™ MR system, offers the promise of expanding MRI studies across LMICs, including Africa, overcoming high costs and infrastructural requirements.[37] These low-cost initiatives are showing results comparable with high-field MRI, and further work is needed to validate whether they are able to replicate the changes in HEU children observed to date.

Structural imaging is one domain of neuroimaging, other modalities include diffusion tensor imaging (white matter microstructure and connecting tracts), magnetic resonance spectroscopy (brain metabolites), resting and active state functional MRI (connectivity), electroencephalography, and near-infrared spectroscopy. Given the reported cortical and subcortical structural differences between HEU and HU children, examining structural and functional networks at the brain-cognition interface is an important next step. In particular, investigating associations between HIV exposure, fronto-striatal connectivity, and white matter pathways will be useful to determine wider network effects, and may explain more

variance than the individual structures alone. The relationship between white matter and inflammation, notably in HIV infection, is well-documented.[38] Studies of people living with HIV have noted changes to connecting frontostriatal white matter,[39] and in HEU children, differences in white matter integrity,[40-42] and associations with inflammatory neurometabolite patterns,[43] have been found. Using magnetic resonance spectroscopy, diffusion tensor imaging, and functional MRI, as well as structural scans, will allow cross-modal analyses to provide greater insight into neurobiological processes and risk stratification.

12.2.3 Investigating the role of child biological sex

Evidence from adults and adolescents has demonstrated sex differences in brain structure,[44-46] with larger brain volumes in males than females in absolute terms,[26] aligning with anthropometric growth differences. A meta-analysis found 8% to 13% larger absolute volumes in males,[45] most notably in total intracranial volume.[47,48] Few studies have examined early childhood, although some have reported sex differences at birth,[44,49,50] and sexual dimorphism in early brain trajectories.[51] However, the size and direction of the differences vary by tissue type and region,[49,52] and within-group cortical morphometry variability has been found to be greater than group differences between sexes.[52,53] These volumetric differences have been hypothesised to be predominantly due to surface area,[54] as reports of global and regional cortical thickness trajectories are reported to be similar in males and females.[54-57] This aligns with prior evidence suggesting variation in surface area may be strongly influenced by sex and genetic factors, while cortical thickness is more impacted by environmental factors.[58-60] Subcortical differences by sex have also been reported;[47,48] notably, the putamen has been reported to be larger in males.[45,47] However, after correcting for brain volume, differences reduce and may largely reflect total brain size differences.[61-63] The clinical significance of these differences remains to be determined.

Few studies have examined sex differences in neurodevelopmental outcomes of children who are HEU.[64] Overall, male children appear to be more susceptible to poorer neurodevelopmental outcomes than female children,[65,66] however, investigations of sex-specific effects of HIV exposure are lacking. In the analysis of general cognitive function at 3.5 years, there was an interaction between sex and HIV exposure and male HEU children were found to have greater cognitive impairment compared to their HU peers. Additionally, sex-dependent associations between neurocognitive outcomes and measures of maternal HIV and ART exposure were also identified (Appendix XI). This builds on earlier research from the DCHS cohort and other settings showing a greater vulnerability of young male children,[66,67] particularly in combination with other environmental and psychosocial risk

factors. Given this evidence, the role of child sex requires further study, alongside understanding the role of biological (hormonal), genetic, psychosocial (for example, differential parental interaction) and environmental factors.

12.2.4 Understanding Causal and Mechanistic Pathways

The neuroimaging findings provide important insights into neural pathways underlying brain (dys)function, however, as this was an observational study questions remain regarding mechanisms and causality. HIV exposure represents a package of potential risk factors for development,[1] including altered immunity/inflammation, ART exposure, poor parental health, and various biological nutritional, environmental and psychosocial factors associated with living in a family affected by HIV. Further research is needed to define causal pathways and mechanisms underlying developmental deficits to develop effective prevention and intervention strategies. As described in the Conceptual Framework model (Chapter 5), mechanisms may be HIV-specific or augmentation of universal risk factor pathways.[68] Another South African study found preterm HEU children are at greater risk for developmental delays compared to those born at term, suggesting a cumulative effect of risk or a high-risk subgroup.[69] The two-hit hypothesis suggests that poor outcomes may result from an early-life priming event that produces vulnerability, followed by an adverse precipitating event or environmental impact.[70] The association between maternal CD4 cell count, brain and language outcomes supports *in utero* inflammation as a priming/sensitising risk.[71] Four potential mechanisms that are currently being investigated in the DCHS and other regional cohorts:

(1) **Inflammation & Immune Activation**. The identified association between smaller grey matter volumes and language metrics with maternal CD4 cell count in pregnancy provides an avenue for exploring immune activation and inflammation as potential mechanisms through which HIV exposure may affect brain structural development and function. This builds on (i) preliminary associations between altered maternal and child inflammatory markers and neurodevelopment in HEU children,[72] and a neurometabolic inflammatory pattern in HEU children in the DCHS (Appendix XII); (ii) observations from other areas that neurodevelopmental changes may manifest through immune-mediated responses which induce aberrant immune programming in the fetal brain, leading to specific brain (anatomy and function) and behaviour phenotypes.[73] Further work to examine associations between neuroinflammatory markers and brain structure in HEU children may guide potential interventions targeting the immune response.

(2) **The role of co-infections.** Co-infections, including CMV, may play a role in determining poor outcomes observed in children who are HEU. Prior research has shown an association between CMV infection, maternal HIV infection, and adverse child outcomes.[74] Similarly, CMV viremia has been found to predict mortality in HEU children,[75] while congenital and early-life CMV infection are thought to influence growth and neurodevelopment, [76] particularly in HIV-exposed children,[77] potentially through immune activation.[78] However, data are limited,[79] and the impact of CMV infection on HEU child brain development requires further investigation. Conversely, exploring the impact of HIV exposure during pregnancy on the developing brain may also provide insight into effects of other infections.

(3) **Anaemia**. Recent DCHS research demonstrates an association between maternal anaemia in pregnancy and smaller child brain volumes, notably in the basal ganglia nuclei and corpus callosum.[80] Anaemia in pregnancy is a well-described risk factor for poor maternal and child outcomes[81-83] and a leading cause of lost developmental potential in LMICs.[84] Anaemia is associated with chronic diseases, including HIV, and iron deficiency is the most common form of anaemia in LMICs.[85] This may affect the dopaminergic neurotransmitter system of the basal ganglia and white matter myelination,[86]and is critical for the developing brain. Supplementation has shown mixed effects suggesting timing and dose are important to consider,[18] and prompting further exploration of the relationship between maternal HIV, iron, and brain development.

(4) **ART regimens.** Neurotoxicity from ART exposure is one plausible mechanism underlying the observed outcomes in HEU children. ARV drugs can cross the placenta with varying degrees of penetration. Although no associations were seen between ART exposure and brain outcomes in this study, it is difficult to disentangle the effects as most mothers were on the same regimen. Potential associations with poorer language outcomes have been reported with exposure to specific ARVs,[87] however, other studies suggest earlier ART initiation with longer exposure is protective, perhaps relating to viral suppression and better immunity.[88] Dolutegravir-based ART is now the recommended first-line regimen in pregnancy. Monitoring the neurodevelopmental outcomes following exposure to both new and old ART regimens remains a priority to determine any adverse effects and clarify the best maternal ART regimen for child outcomes.

12.2.5 Extrapolating findings within the wider developmental literature

Finally, the findings may be extrapolated to the wider developmental literature through exploring thematic neuroanatomical signatures of childhood developmental disorder phenotypes. The brain is particularly vulnerable to genetic and environmental exposures during early development.[89,90] Deviations from the set neurodevelopmental trajectory are thought to underlie many childhood disorders,[91] including autism spectrum disorder (ASD), which has been hypothesised to manifest as a consequence of altered brain development in the rapid brain growth stage during infancy.[91,92] Interestingly, ASD and other neurodevelopmental disabilities have a key language and communication aspect. Language delay is a frequent early precursor to ASD and is an indicator of ASD by age two.[93] Pre-school language delay has separately been found to predict ASD classification in association with brain structural abnormalities (e.g. isolated fetal ventriculomegaly).[94] As a result, monitoring language may provide an opportunity for early identification and targeted support for the most at-risk children, and possibly a biomarker of later cognitive function, behaviour or risk of developmental disorders.[95]

The neuroimaging results have parallels with neuroanatomical findings reported in studies of typically developing children where infant brain volumetric variability has been associated with behavioural outcomes.[96] In studies of children with neurodisabilities, symptoms are also often accompanied by structural brain differences. In particular, volumetric differences in deep grey matter in older children have been related to various disorders.[97,98] Altered fronto-striatal connectivity, alongside frontal and striatal volumetric changes, represent elements of the ASD executive function deficit theory, which is associated with developmental trajectory differences.[99-101] Mega-analysis results from the ENIGMA Consortium found that ASD was associated with smaller subcortical volumes of the pallidum, putamen, amygdala and nucleus accumbens, as well as increased cortical thickness in the frontal cortex (and reduced temporal cortex) with few or no differences in surface area.[102] Other studies have shown a similar picture of greater cortical thickness in frontal lobes compared to controls, and atypical cortical thickness with striatal volumetric changes are some of the most replicated features.[103,104] Separately, studies of children with attention deficit hyperactivity disorder have found abnormal frontostriatal circuits and delay in cortical thickness maturation, particularly in the prefrontal cortex.[105]

In line with this literature, the thesis results suggest that specific neural patterns or altered cortical maturation trajectories - 'neurobiological signatures' - may translate into specific phenotypes. In turn these signatures may be associated with certain risk factors. However,

with this in mind, it is important to acknowledge both equifinality – that diverse pathways may result in the same outcome, and multifinality – that various outcomes may occur from the same pathways in different individuals by changing one component.[106,107] Given neurobiological processes underlying neuroanatomical development are regulated through many environmental and genetic factors,[108] this aligns with the multi-hit hypothesis, that neurodisability phenotypes may result from multiple adverse factors or events.[19] Identification of early biomarkers or deviations from typical maturation trajectories offers the potential for earlier interventions that take advantage of brain plasticity in infancy and the design of targeted pre-emptive intervention strategies to prevent disorder onset.

12.3 Community Engagement

12.3.1 Family-centric approach

While the focus of prevention strategies is angled more towards mothers/caregivers, and the focus of intervention strategies is directed more towards the children, it is important that both intervention and prevention strategies involve families as a whole and the surrounding community. Taking family-centric approaches that reduce stigma are vital. Further, educating on maternal and child health during pregnancy and post-partum, and informing parents about early identification, and simple interventions to improve neurodevelopment will empower parents.

12.3.2 Understanding community perspectives and public engagement

Community and public engagement are key aspects of this work and care needs to be taken when communicating the results of the research to ensure they are conveyed accurately, sensitively, and appropriately. As such, involving members of the community in the discussion and considering the context is critical. In this final section, public engagement work that took place as a result of this thesis on the topic of neurodevelopment is described.

In collaboration with colleagues and the DCHS social responsiveness team with funding from LSHTM, we designed and ran the two interactive workshops titled '*Neuroimaging in focus: engaging with mothers around child brain development*' in Paarl, South Africa. The workshops aimed to engage mothers in the DCHS around neuroimaging and child brain development. The objectives were:

(i) To explore participant experience of the neuroimaging and identify ways to improve the research processes going forward;

(ii) To understand community perception of child development and associated factors;

(iii) To share study findings in an interactive format that empower caregivers to promote healthy brain development in the local community;

(iv) To work together to identify key child development messages and discuss approaches to sharing study outcomes with the wider community; and

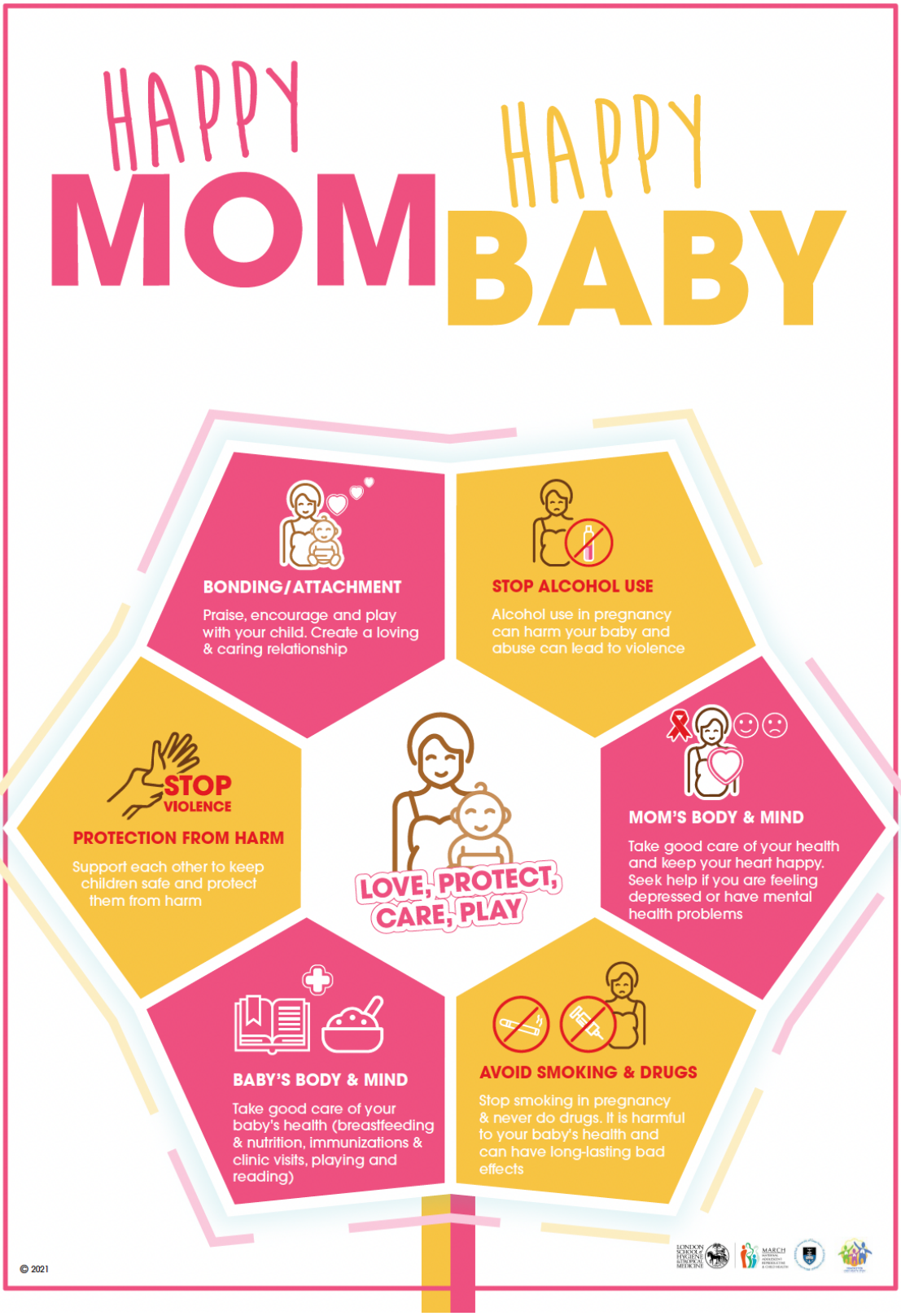
(v) To develop a strategy to enhance two-way communication and dialogue with the community.

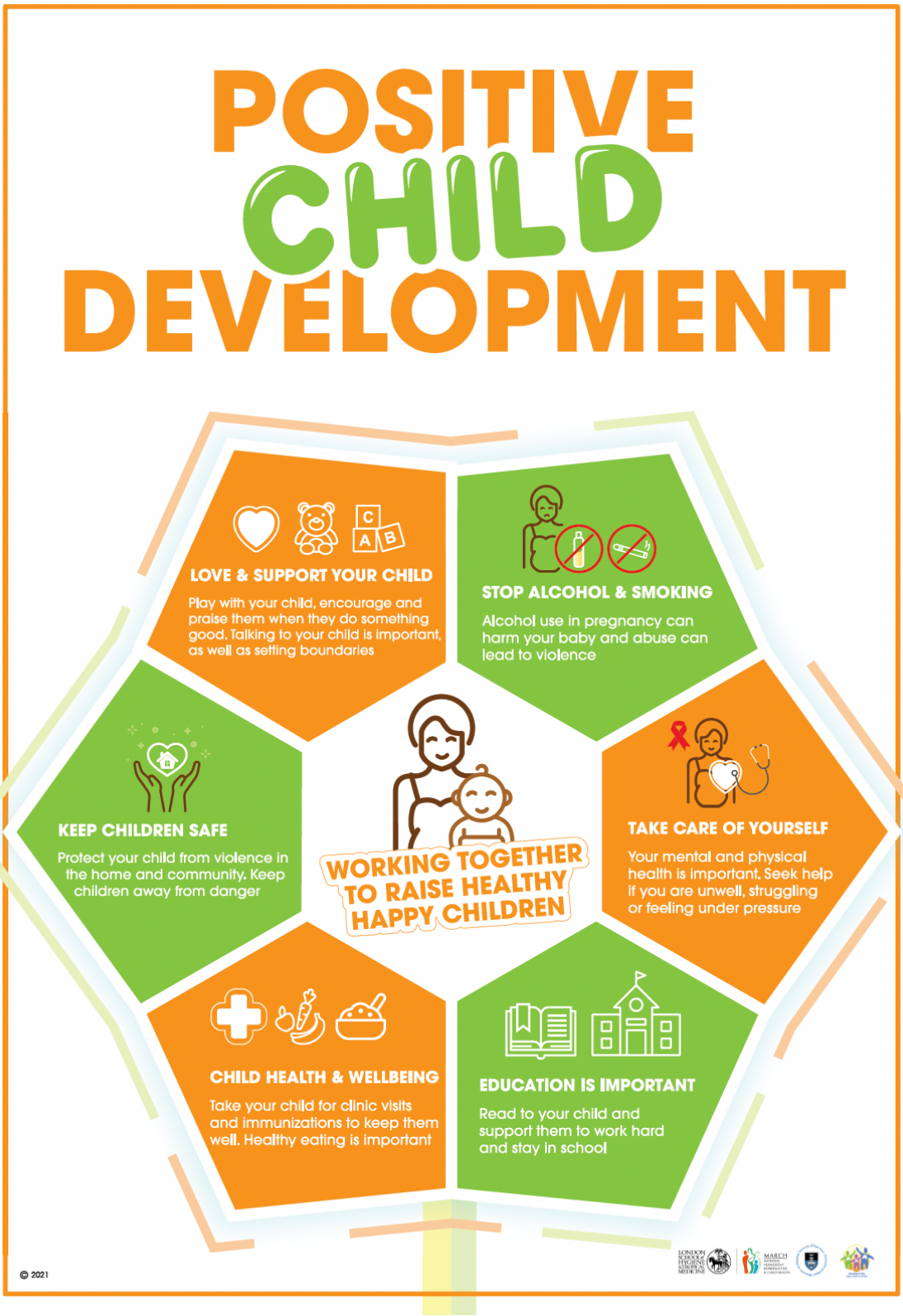
The objectives were achieved through interactive presentations and roundtable discussions. A set of key themes that are important for child development were agreed upon based on community knowledge and study findings, these included: safety; caring for the mother's physical and mental health; avoiding alcohol, smoking, and drugs in pregnancy; child health, education and nutrition; supporting and nurturing children. Maternal HIV infection was discussed as part of maternal health, and ART adherence was understood to be important for child well-being. Participants then created posters during the workshops based on the themes which were used to design two combined digital posters to share with the wider study community (Figure 12.1).

Participant experience of the neuroimaging was positive and gave insight into community views on the neuroimaging which have been used to inform and enhance future neuroimaging directions and DCHS community engagement. Mothers highlighted the friendly staff; clear scan explanation; children feeling safe, comfortable, and enjoying the experience; and taking a photo of the scan home. Some processes were identified as being important to the mothers including: safe and convenient transport with advance bookings; the value of an on-site translator; and providing meals for both the mother and child. Participant suggestions were incorporated into future study processes.

Overall, the workshops were able to improve understanding of child development as well as research processes. Through working together, community priorities for child development were identified and two-way knowledge translation between the study and the community was strengthened with practical approaches to promote healthy brain development.

Figure 12.1: Neuroimaging in focus workshop output: posters to promote optimal early child development




POSITIVE CHILD DEVELOPMENT
LOVE & SUPPORT YOUR CHILD
Play with your child, encourage and praise them when they do something good. Talking to your child is important, as well as setting boundaries
STOP ALCOHOL & SMOKING
Alcohol use in pregnancy can harm your baby and abuse can lead to violence
KEEP CHILDREN SAFE
Protect your child from violence in the home and community. Keep children away from danger
WORKING TOGETHER TO RAISE HEALTHY HAPPY CHILDREN
TAKE CARE OF YOURSELF
Your mental and physical health is important. Seek help if you are unwell, struggling or feeling under pressure
CHILD HEALTH & WELLBEING
Take your child for clinic visits and immunizations to keep them well. Healthy eating is important
EDUCATION IS IMPORTANT
Read to your child and support them to work hard and stay in school
© 2021

12.4 Overall Conclusion

Uninfected but still affected:
Neurodevelopmental effects of HIV exposure

Widespread success in prevention of vertical transmission coupled with an ongoing high antenatal HIV prevalence in many SSA countries has resulted in a growing population of children born HIV-exposed who remain HIV-free, estimated at over 16 million.[109] This thesis presents evidence on the early neurodevelopment of children who are HEU in a South African context. Overall, the research adds to our understanding of the vulnerability of HEU children, notably in early language development. Language skills are foundational for school readiness and are important predictors for later literacy and executive function. Therefore, subtle deficits herald concerns for future school and academic attainment at older ages, with implications through adulthood.[110] The results indicate that HIV exposure can affect structural brain development, particularly in frontostriatal regions, which may be a pathway leading to language deficits reported in children who are HEU. Identification of brain structure-function relationships expands existing knowledge of neuroanatomical pathways underlying language development. The association of antenatal HIV exposure with early brain structure suggests that changes may happen *in utero* and persist, potentially indicating delayed or altered brain maturation of specific brain regions. These findings indicate that careful developmental monitoring of children who are HEU is warranted. The association of child outcomes with maternal CD4 cell count and viral load in pregnancy indicates the importance of optimising antenatal maternal HIV management to mitigate poor child outcomes and to consider the role of immune activation in brain development. With over one million children born each year with HIV exposure, it is important for healthcare workers in areas with high HIV prevalence to be aware of this growing vulnerable population and to engage with the community to improve outcomes. While evidence suggests early developmental differences, further research is needed to determine the long-term outcomes and to define the underlying mechanisms that will inform prevention and intervention strategies to ensure children who remain HIV-free also thrive.

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Appendices

Appendix I: Book chapter publication rights agreement

Book title and edition ("the Book")	Recent Advances in the Neurological and Neurodevelopmental Impact of HIV
Book Editor(s) ("the Editor")	Amina Abubakar, Kirsty Donald, Jo Wilmshurst, and Charles Newton
Chapter title/s ("the Contribution")	Chapter 15, 'Neurodevelopment of children who are HIV-exposed and uninfected'
Chapter author(s) ("the Contributor")	Catherine J Wedderburn, Shunmay Yeung, and Kirsten A Donald

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Appendix II: Summary of systematic reviews and meta-analyses on neurodevelopment of children who are HEU

Articles are listed in chronological order. Studies in white represent evidence available at the start of the thesis to provide rationale for the work, while studies in blue represent research at/after this thesis, including a paper from the thesis. Table adapted from *Recent Advances in the Neurological and Neurodevelopmental Impact of HIV.* Editors: Amina Abubakar, Kirsten A Donald, Jo M Wilmshurst, Charles R Newton. Mac Keith Press 2023.

Title, Author, Year	Studies included	Region (*N* studies)	Findings
A systematic review of psychological functioning of children exposed to HIV: using evidence to plan for tomorrow's HIV needs (Sherr et al, 2014) [1]	11 studies	Africa (6) Asia (1) Europe (1) North America (3)	• 7/11 studies reported CHEU performed lower in at least one psychological measure when compared to HUU children spanning cognitive, developmental and behavioural parameters. However, findings differed across studies. (i) Cognitive: Overall IQ scores were lower in pre-school CHEU compared to CHU, however, mixed results were reported across countries. Lower scores for reading ability and language expression were reported in CHEU from studies in Africa, but not the USA. (ii) Motor development: Some studies found CHEU had poorer motor development, while others did not. (iii) Behaviour: Varying reports of worse externalising and internalising behaviour in pre-adolescents who are HEU across countries.
Clinical outcomes of HIV-exposed, HIV-uninfected children in sub-Saharan Africa (Le Roux et al, 2016) [2]	8 studies contributed 9 reports assessing early child development outcomes. Of these, 4 studies (5 reports) had low/moderate risk of bias with varying maternal ART use (n=1 mothers received triple ART regardless of disease staging; n=2 mothers received ARVs for PMTCT; n=1 mothers received no antiretrovirals).	All sub-Saharan Africa	• Limited studies from the current PMTCT era. • No difference was reported in preschool development in CHEU compared to CHU, or in 6-12 year old CHEU in the Democratic Republic of the Congo (DRC). • Lower educational achievement among school-age CHEU in Zambia compared to CHU, as measured by lower mathematics grades.
Neurodevelopment in Young Children Born to HIV-Infected Mothers: A Meta-analysis (McHenry et al, 2018) [3]	45 studies (46 cohorts) were included in the review examining children with HIV, HEU, and HUU. Meta-analysis included 4 studies that compared HEU and HUU children from Africa (n=2), South America (n=1), North America (n=1).	Africa: (18) Asia (1) Europe (4) North America (20) South America (3)	• Systematic review: CHEU were described as having worse developmental outcomes compared with CHU in a few studies; children living with HIV had worse outcomes than comparison groups in many studies. • Meta-analysis: CHEU had lower mental developmental index (cognitive) and psychomotor developmental index (motor) scores compared to CHU peers up to age eight years, although to a lesser extent than children living with HIV. • Studies outside the USA received a low-quality rating. In studies limited to high-quality assessments, CHEU had similar scores to CHU. • CHEU with antiretroviral (ARV) exposure had lower cognitive and motor scores than those without ARV exposure.

In utero exposure to HIV and/or antiretroviral therapy: a systematic review of preclinical and clinical evidence of cognitive outcomes (McHenry et al, 2019) [4]	5 studies of children (0-15 years) examining brain structure and function. A further 20 studies focused on behavioural assessments in animal models.	Africa (2) Asia (1) Europe (1) South America (1)	• Overall few clinical studies. • Four studies used magnetic resonance imaging: two reported differences on diffusion tensor imaging between CHEU and CHU aged 2-6 weeks and 7 years; one found no group differences at mean age of 10 years; a final study examined individual qualitative outcomes of structural MRI of CHEU at 10-44 months presenting with neurologic symptoms and found 50% of children had MRI abnormalities. • Another study found exposure to zidovudine +/- lamivudine may affect brainstem auditory evoked potentials in HEU infants.
In Utero HIV Exposure and the Early Nutritional Environment Influence Infant Neurodevelopment: Findings from an Evidenced Review and Meta-Analysis (White and Connor, 2020) [5]	24 studies: 15 studies assessed neurodevelopmental outcomes from birth to 36 months in infants born to mothers living with HIV; 9 studies reported on early life nutrition-related variables and infant neurodevelopment. Meta-analysis included 3 studies (4 articles) examining BSID-III sub-scales for infants who are HEU compared to HUU (2 Africa, 1 South America).	Africa (17) Asia (2) Europe (2) South America (2) North America (1)	• Review: HIV exposure was associated with lower scores in some studies, and child HIV infection with lower scores in many studies. • Meta-analysis: expressive language function was worse in CHEU compared to CHU in the first three years; other domain estimates were lower in CHEU but did not show significant differences. • Male infants were found to be more vulnerable to HIV exposure, although few studies specifically examined sex-dependent effects. • Maternal perinatal micronutrient supplementation improved motor outcomes at six months in CHEU. Infant supplementation alone did not affect neurodevelopment. Nutrient supplementation and education combined with a water, sanitation, and hygiene intervention improved neurodevelopment at 24 months in CHEU.
Early neurodevelopment of HIV-exposed uninfected children in the era of antiretroviral therapy: a systematic review and meta-analysis (Wedderburn et al, 2022) [6]	31 studies (45 articles): 21 studies compared neurodevelopment between HEU and HUU children; 10 studies examined ART regimens; further, 13 studies reported on head circumference or neuroimaging. Meta-analysis included 8 studies (7 Africa; 1 North America) and a total of 1856 HEU and 3067 HUU children.	Africa (22) South America (3) North America (4) Asia (2)	• Systematic review: 57% of studies found worse neurodevelopment among CHEU in at least one domain compared to CHU. • Meta-analysis: at 12–24 months of age, on average CHEU had poorer expressive language and gross motor development than CHU. • Few studies examined ART. Limited evidence suggests little, if any, impact of specific maternal ART regimens on neurodevelopment, although concerns were raised regarding atazanavir and efavirenz. • Larger studies suggested CHEU may have smaller head circumferences in early life compared to CHU. There was only one neuroimaging study of children under 5 years.

Abbreviations: CHEU: children who are HIV-exposed uninfected; CHU: Children who are HIV-unexposed

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Appendix III: Narrative summary of neuroimaging studies of children who are HEU

Articles are listed in chronological order. Studies in white represent evidence available at the start of the thesis to provide rationale for the work, while studies in blue represent research at/after this thesis, including papers from the thesis itself to put them in the context of current literature.

Title, Author, Year	Study name; Recruitment period	Country	Study groups (n)	Age	Imaging modality	Findings
Cerebral MR imaging in uninfected children born to HIV-seropositive mothers and perinatally exposed to zidovudine (Tardieu et al, 2005)[1]	No study period given. Note: 13 exposed to zidovudine (AZT) 19 to AZT + lamivudine dual therapy	France	49 CHEU	2 years	Structural Imaging (qualitative)	16/22 images of children with mitochondrial dysfunction were abnormal; 8/27 images of children without mitochondrial dysfunction were abnormal.
Brain imaging and neurodevelopment in HIV-uninfected Thai children born to HIV-infected mothers (Jahanshad et al, 2015)[2]	PREDICT study (2006 – 2011) Note: 14/30 HEU children born before year 2000 with no ART exposure.	Thailand and Cambodia	30 CHEU 33 CHU	10 years	Structural & Diffusion Tensor Imaging	No group differences detected. FA and MD associated with IQ scores.
White matter microstructural integrity and neurobehavioural outcome of HIV-exposed uninfected neonates (Tran et al, 2016)[3]	Drakenstein Child Health Study (2012 – 2015)	South Africa	15 CHEU 22 CHU	Neonates	Diffusion Tensor Imaging	Higher FA in middle cerebellar peduncles in CHEU compared to CHU; correlation between neurobehavior and FA in uncinate fasciculus CHEU.
Longitudinal increases of brain metabolite levels in 5-10 year old children (Holmes et al, 2017)[4]	Longitudinal multimodal neuroimaging study	South Africa	11 CHEU 12 CHU	5 – 10 years	Magnetic Resonance Spectroscopy	No age-related differences in metabolite trajectories by HIV exposure.
White Matter Abnormalities in Children with HIV Infection and Exposure (Jankiewicz et al, 2017)[5]	CHIV from CHER study 2005 - 2007 CHEU and CHU from a vaccine study enrolled 2005 - 2006 at 6-12 weeks	South Africa	65 CHIV 19 CHEU 27 CHU	7 years	Diffusion Tensor Imaging	Higher FA and lower MD in CHEU compared to CHU in corona radiata and corticospinal tracts respectively
Perinatal HIV infection or exposure is associated with low N-acetylaspartate and glutamate in basal ganglia at age 9 but not 7 years (Robertson et al, 2018)[6]	CHER study (2005 - 2007) Vaccine study (2005 - 2006)	South Africa	37 CHIV 10 CHEU 13 CHU (both timepoints)	7 and 9 years	Magnetic Resonance Spectroscopy	No difference at 7 years. At 9 years, CHEU had lower NAA and Glu, as well as lower creatine and choline in the basal ganglia compared to CHU.
Brain microstructural changes support cognitive deficits in HIV uninfected children born to HIV infected mothers (Yadav et al 2020)[7]	Recruited 2011 – 2013 aged 10-12 years. NB: Half CHEU born <2000 with no ART details; 11 born 2000 -2003 to mothers on AZT	India	49 CHIV 8 CHEU 18 CHU	10-12 years	Diffusion Tensor Imaging	Decreased FA and altered MD in multiple regions in CHEU compared to CHU; correlations with neuropsychological scores
MRS suggests multi-regional inflammation and white matter axonal damage at 11 years following perinatal HIV infection (Graham et al, 2020)[8]	CHER study (2005 – 2007) Vaccine study (2005 - 2006)	South Africa	76 CHIV 30 CHEU 30 CHU	11 years	Magnetic Resonance Spectroscopy	Reduced NAA in peritrigonal white matter in CHEU compared to CHU, alongside lower absolute NAA and Glu in the mid-frontal grey matter.

Altered white matter tracts in the somatosensory, salience, motor, and default mode networks in 7-year-old children living with Human Immunodeficiency Virus: a tractographic analysis (Madzime et al, 2021)[9]	CHER study (2005 - 2007) Vaccine study (2005 - 2006)	South Africa	61 CHIV 19 CHEU 27 CHU	7 years	Diffusion Tensor Imaging	Altered white matter integrity (higher FA) in the visual, ventral default mode, and motor networks in CHEU compared to CHU.
A neurometabolic pattern of elevated myo-inositol in children who are HIV-exposed and uninfected: a South African birth cohort study (Bertran Cobo et al, 2022)[10]	Drakenstein Child Health Study (2012 – 2015)	South Africa	36 CHEU 47 CHU	2-3 years	Magnetic resonance spectroscopy	CHEU has an inflammatory neurometabolic pattern dominated by myo-inositol across parietal grey and white matter. Higher myo-inositol: creatinine ratio in left and right parietal white matter of CHEU versus CHU.
Early structural brain development in infants exposed to HIV and antiretroviral therapy in utero in a South African birth cohort (Wedderburn et al, 2022)[11]	Drakenstein Child Health Study (2012 – 2015)	South Africa	40 CHEU 106 CHU	2- 6 weeks	Structural Imaging (T2-weighted)	Smaller total grey matter and caudate (basal ganglia) volumes in CHEU; associations between brain volumes and maternal CD4 count in pregnancy
Maternal ART throughout gestation prevents caudate volume reductions in neonates who are HIV exposed but uninfected (Ibrahim et al, 2023)[2]	Healthy Baby study	South Africa	79 CHEU 41 CHU	Mean gestational age 41.5 +/- 1 weeks	Structural Imaging (T2-weighted)	Smaller left putamen volume in CHEU versus CHU; smaller caudate volumes in CHEU of mothers starting ART post-conception versus pre-conception
Association of *in utero* HIV exposure with child brain structure and language development: a South African birth cohort study (Wedderburn et al, 2023)[3]	Drakenstein Child Health Study (2012 – 2015)	South Africa	70 CHEU 92 CHU	2-3 years	Structural Imaging (T1-weighted)	Increased cortical thickness of prefrontal regions in CHEU (mOFC); correlation between cortical thickness and language development; mediation
Subcortical brain volumes and neurocognitive function in children with perinatal HIV exposure: a population-based cohort study in South Africa (Wedderburn et al, 2024)[14]	Drakenstein Child Health Study (2012 – 2015)	South Africa	70 CHEU 92 CHU	2-3 years	Structural Imaging (T1-weighted)	Smaller subcortical volumes in CHEU, notably putamen (basal ganglia) and hippocampus; associations with maternal HIV severity and child language

Legend: To identify relevant studies, a literature search was performed in Pubmed and Scopus including the terms ((infant OR child) AND (HIV AND expos*AND uninfected) AND (neuroimaging OR brain structure)). References from the review by McHenry MS *et al* 'In utero exposure to HIV and/or antiretroviral therapy: a systematic review of preclinical and clinical evidence of cognitive outcomes,[3] were also reviewed.

Abbreviations: CHEU: children who are HIV-exposed uninfected; CHU: Children who are HIV-unexposed; NAA: N-acetyl-aspartate; Glu: glutamate; FA: fractional Anisotropy; MD: Mean Diffusivity

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Appendix IV: Data Management Plan

Data management principles

The study will adhere to strict data management procedures established by the Drakenstein Child Health study (DCHS), LSHTM Research Data Management Policy and forthcoming University of Cape Town (UCT) RDM Policy. Security processes will be in place with access permissions to protect the data and these will comply with UCT and LSHTM information security policy.

Participant data will be collected and managed in an ethical manner in accordance with research ethics committee stipulations, maintaining confidentiality at all times and adhering to Good Clinical Practice principles, the latest version of the Declaration of Helsinki, and the South African Department of Health: Ethics in Health Research principles, structures and processes. Informed consent is taken at enrolment and renewed annually as part of the DCHS, and includes data sharing provisions. Informed consent will also be taken before each imaging session from a parent/legal guardian.

Data description

The proposed study will involve collecting the following data from neuroimaging study participants:

1. Neuroimaging data (MRI, DTI) collected during scanning procedures;
2. Neurodevelopmental assessment outcomes collected at research visits;
3. Anthropometric measurements and clinical data collected during research visits and clinical data from folder review.

Access to the following data that have been captured as part of the broader DCHS will also be available to be cleaned and used in the analyses:

4. Sociodemographic, environmental, nutritional, psychosocial, and health-related information on study participants and their families generated from questionnaires;
5. Clinical data on mothers of study participants relating to HIV testing and treatment collected from folder review;
6. Neurodevelopmental assessment measure outcomes from age 2-4 weeks, 6 months, and 2 years from across the wider study.

Data collection and capture

DCHS background information and anthropometric measurements are recorded on paper and then uploaded electronically to a RedCAP database. In the future the annual DCHS visits will use electronic data capture platforms which are password protected. Imaging data are electronic and the source images will be automatically uploaded and stored before processing. Neurodevelopmental (BSID-III) assessments are recorded on paper and then uploaded into the Bayleys software tool and exported to RedCAP. Neurocognitive assessments are captured directly electronically. All data will be anonymised from the point of collection and linked by unique participant identifiers. Personal information will be removed from the dataset and stored in a different location, using a unique identifier as a link.

Data analysis

Output 2 will be cleaned and stored in a RedCAP database and exported to the most recent version of STATA for statistical analysis. Images will be processed by FSL (FMRIB Software Library) or alternative appropriate imaging programmes, and the processed numerical output will be analysed in excel or STATA.

Data storage:

- Electronic data generated by the DCHS will be stored on RedCap in a UCT-based server (the Drakenstein Child Health data repository, not open to the public) managed by the data manager with regular quality checks (back-ups and integrity monitoring) and access permissions. The Drakenstein data repository will be maintained for at least 10 years following study completion.
- Neuroimaging data will be stored on a UCT server network drive automatically, and a copy will also be stored in the Drakenstein data repository.
- Paper-based data (consent forms, neurodevelopmental assessments, Drakenstein background information) will be stored in a secure locked location in a UCT office only accessible to study staff.
- Sensitive data will be kept separate to other data. Cleaned anonymised data without sensitive information will be kept on personal laptops and external hard drives to perform analysis. Any data on personal computers will be encrypted.
- Back-ups will be performed regularly.

Data quality

Quality checks will be performed regularly during collection and upload to look for information that is incomplete due to incorrectly completed forms and clinical folder information, and to document missing information from unsuccessful scans. Data quality will be maintained by conducting data checks periodically on a subset of data verifying against source and integrity. Imaging data will be appraised by image review, blinded to HIV status, any scans that fail to meet study quality standards will be discarded from the analysis. Neurodevelopmental data will be checked at site and then centrally for scoring errors.

Documentation and metadata

A data dictionary is available for DCHS study codes which will be adapted for this study.

Data organization

Data will be stored as a set of files including a source file and subsequent labelled and dated versions following any processing.

Data security

- Paper-based records will be kept in a secure controlled location only accessible to authorized personnel involved in the study;
- Computer-based records will only be available to authorised users involved in the study using access privileges and passwords;
- Personnel will be required to sign statements agreeing to protect the security and confidentiality of identifiable information;
- Personal identifiers will be removed from research-related information;

- Encryption will be used to store data and transfer data, as well as password protection;
- Other. Please specify: *See section on data collection and capture.*

Data archiving and sharing

Data captured by the DCHS and this research proposal may have benefit to the wider research community, providing insight into global child health. UCT and LSHTM are committed to the principle of data sharing. Data will be made available in compliance with Drakenstein study policy. Open and widely-documented formats will be used where feasible to ensure data remains accessible in the long-term and this will be investigated at a future date. Following study completion, secondary data will be held by the DCHS in the Drakenstein data repository so that further longitudinal analysis is possible. An anonymised copy of this study data will be stored on the PI's personal drive for the purpose of analysis and publication.

1. *Sharing with the wider research community:* De-identified data will be made available to external researchers on request. A data access agreement specific to this study will be developed. Requests may be made by completing a one-page application form submitted to the DCHS Principal Investigator and the technical expert documenting the data requested, what the data will be used for, and the research aims. A data sharing agreement will be signed by anyone requesting data to ensure the need to maintain confidentiality of the information and protect against misuse. When publications are written, researchers will be directed towards the data request process if needed. Research papers will adhere to the Wellcome Trust's open access policy.
2. *Sharing with policy-makers:* Results from the work will be submitted as abstracts to meetings for presentations and for publishing in open-access journals.
3. *Sharing within the Drakenstein study:* Anonymised data will be freely shared within the Drakenstein study group.

To action this plan, LSHTM IT, RDM support and data management teams will be consulted for advice and relevant training, for example RedCAP and metadata creation.

Appendix V: Supplementary Information Chapter 4

Neurodevelopment of HIV-Exposed Uninfected Children in South Africa: Outcomes from an Observational Birth Cohort Study *(research paper)*

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Wedderburn CJ, Yeung S, Rehman AM, et al. Neurodevelopment of HIV-exposed uninfected children in South Africa: outcomes from an observational birth cohort study. *Lancet Child Adolesc Health* 2019; published online Sept 9. http://dx.doi.org/10.1016/S2352-4642(19)30250-0.

Appendix 1: Antiretroviral drug regimens received by HIV-infected mothers during pregnancy

Antiretroviral drug (ARV) regimen	BSID-III timepoint	
	6 months HIV-infected mothers* *N*, %	**24 months HIV-infected mothers*** *N*, %
PMTCT prophylaxis (AZT)	9/58 (16%)	20/163 (12%)
1st line three-drug antiretroviral therapy (ART)		
TDF + (3TC or FTC) + EFV (separately or fixed dose combination)	42/58 (72%)	112/163 (69%)
AZT + 3TC + EFV	-	3/163 (2%)
d4T + 3TC + EFV	-	1/163 (1%)
TDF + 3TC + NVP	4/58 (7%)	9/163 (6%)
AZT + 3TC + NVP	2/58 (3%)	6/163 (4%)
d4T + 3TC + NVP	-	1/163 (1%)
2nd/3rd line ART		
AZT + 3TC + LPV/r	1/58 (2%)	6/163 (4%)
TDF + 3TC + LPV/r	-	4/163 (2%)
AZT+ 3TC + RIT + ATV	-	1/163 (1%)

Footnotes:
Abbreviations: ART = Antiretroviral therapy; AZT = zidovudine; EFV = efavirenz; 3TC = lamivudine; FTC = emtricitabine; TDF = tenofovir; d4T = stavudine; NVP = nevirapine; LPV/r = lopinavir/ritonavir (Kaletra); RIT = ritonavir; ATV = atazanavir
* missing data at 6 months n =3; at 24 months n=5. Percentages are cited among those with non-missing values.

Appendix 2: Comparison of demographics among children who had a BSID-III assessment measured at either time-point and those who were in follow up but did not have a BSID-III.

Variables	6 months			24 months		
	BSID-III 260 (24·4%)	No BSID-III 805 (75·6%)	*P*	BSID-III 732 (73·2%)	No BSID-III 268 (26·8%)	*P*
HIV exposure	61/260 (23%)	170/805 (21%)	0.43	168/732 (23%)	51/268 (19%)	0.18
Male sex	135/260 (52%)	410/805 (51%)	0.78	378/732 (52%)	135/268 (50%)	0.72
Site (Mbekweni)	127/260 (49%)	461/805 (57%)	0.018*	388/732 (53%)	155/268 (58%)	0.17
Monthly household income (ZAR)						
< R1000 (<~$75)	114/260 (44%)	290/805 (36%)	0.056	287/732 (39%)	99/268 (37%)	0.81
R1000-R5000 (~$75-375)	119/260 (46%)	403/805 (50%)		356/732 (49%)	135/268 (50%)	
>R5000 (>~$375)	27/260 (10%)	112/805 (14%)		89/732 (12%)	34/268 (13%)	
Maternal education						
Primary	15/260 (6%)	61/805 (8%)	0.44	59/732 (8%)	13/268 (5%)	0.38
Secondary	149/260 (57%)	430/805 (53%)		398/732 (54%)	151/268 (56%)	
Completed secondary	84/260 (32%)	261/805 (32%)		233/732 (32%)	87/268 (32%)	
Any tertiary	12/260 (5%)	53/805 (7%)		42/732 (6%)	17/268 (6%)	
Maternal employment status (employed)	58/260 (22%)	228/805 (28%)	0.057	183/732 (25%)	81/268 (30%)	0.097
Relationship status (married / cohabitating)	95/259 (37%)	330/805 (41%)	0.22	292/731 (40%)	101/268 (38%)	0.52
Maternal age at birth, years, mean (SD)	26·5 (5·6)	27·1 (5·7)	0.13	27·3 (5·8)	26·3 (5·3)	0.017*
Gestational age at delivery, weeks, mean (SD)	38·7 (2·2)	38·4 (2·7)	0.17	38·6 (2·5)	38·4 (2·6)	0.25
Prematurity (<37 weeks)	33/260 (13%)	137/801 (17%)	0.092	104/730 (14%)	52/266 (20%)	0.042*
Birthweight (g), mean (SD)	3057 (540)	3029 (604)	0.50	3035 (580)	3046 (594)	0.80
Birth length (cm), mean (SD)	49.7 (3.8)	49.8 (3.8)	0.80	49.9 (3.7)	49.6 (3.7)	0.24
Birth head circumference (cm), mean (SD)	33.5 (1.9)	33.6 (2.1)	0.75	33.6 (2.1)	33.5 (2.0)	0.60
Low birthweight (<2.5kg)	32/260 (12%)	128/805 (16%)	0.16	107/732 (15%)	41/268 (15%)	0.79
Maternal smoking during pregnancy[a]						
Active	101/256 (39%)	232/772 (30%)	0.021*	250/714 (35%)	69/254 (27%)	0.067
Passive	102/256 (40%)	358/772 (46%)		308/714 (43%)	126/254 (50%)	
Non-smoker	53/256 (21%)	182/772 (24%)		156/714 (22%)	59/254 (23%)	
Maternal alcohol use during pregnancy[b]	52/247 (21%)	74/696 (11%)	<0.0001*	95/655 (15%)	21/233 (9%)	0.033*
Maternal depression[c]	66/246 (27%)	158/700 (23%)	0.18	156/657 (24%)	56/233 (24%)	0.93
Exclusive breastfeeding duration, months mean (SD)	2·1 (2·1)	2·1 (1·9)	0.96	2·2 (2·0)	2·0 (1·9)	0.22
Exclusive breastfeeding for 6 months	44/260 (17%)	123/782 (16%)	0.65	124/731 (17%)	34/261 (13%)	0.14
HIV/ARV-related variables (HEU only)						
Maternal HIV diagnosis timepoint						
Before pregnancy	44/59 (75%)	116/163 (71%)	0.62	122/163 (75%)	30/47 (64%)	0.14
During pregnancy	15/59 (25%)	47/163 (29%)		41/163 (25%)	17/47 (36%)	
Maternal CD4 cell count[d]						
Median (range) (cells/mm^3)	522 (298-691)	414 (293-575)	0.088	441 (294-618)	455 (315-635)	0.87

<350 cells/mm^3	18/56 (32%)	59/152 (39%)	0.11	54/151 (36%)	16/45 (36%)	0.999
350-500 cells/mm^3	9/56 (16%)	38/152 (25%)		33/151 (22%)	10/45 (22%)	
>500 cells/ mm^3	29/56 (52%)	55/152 (36%)		64/151 (42%)	19/45 (42%)	
Maternal Viral load (VL) in pregnancy[e]						
Lower than detectable limit (<40 copies/mL)	25/36 (69%)	64/103 (62%)	0.71	69/108 (64%)	16/25 (64%)	0.24
VL detectable (>=40-1000 copies/mL)	6/36 (17%)	23/103 (22%)		25/108 (23%)	3/25 (12%)	
Virally unsuppressed (>1000 copies/mL)	5/36 (14%)	16/103 (16%)		14/108 (13%)	6/25 (24%)	
Antiretroviral drug initiation						
Before pregnancy	22/59 (37%)	73/167 (44%)	0.39	71/165 (43%)	19/49 (39%)	0.60
During pregnancy	37/59 (63%)	94/167 (56%)		94/165 (57%)	30/49 (61%)	
Antiretroviral regimen during pregnancy						
PMTCT prophylaxis (AZT [zidovudine])	9/58 (16%)	23/165 (14%)	0.49	20/163 (12%)	10/49 (20%)	0.30
1st line Triple therapy (non-EFV)	6/58 (10%)	17/165 (10%)		16/163 (10%)	6/49 (12%)	
1st line Triple therapy (EFV-containing)	42/58 (72%)	113/165 (68%)		116/163 (71%)	32/49 (65%)	
2nd/3rd line	1/58 (2%)	12/165 (7%)		11/163 (7%)	1/49 (2%)	
Infant prophylaxis						
NVP [nevirapine] alone	55/60 (92%)	142/167 (85%)	0.19	145/167 (87%)	42/50 (84%)	0.61
NVP + AZT	5/60 (8%)	25/167 (15%)		22/167 (13%)	8/50 (16%)	

Footnotes:
Abbreviations: VL = viral load; NVP = nevirapine; AZT = zidovudine; EFV = efavirenz;

*p<0.05; Unpaired t-test used for continuous variables (means and SD presented); Chi-squared for categorical variables (n and % proportions presented).

Missing data at both timepoints: relationship status (n=1), maternal age at birth (n=1); gestation at delivery (n=4); smoking (n=37 at 6 months, n=32 at 24 months), Alcohol in pregnancy (n=122 at 6 months, n=112 at 24 months), depression (n=119 at 6 months, n=110 at 24 months), breast feeding (n=23 at 6 months, n=8 at 24 months); birth head circumference (n=15 at 6 months; n=14 at 24 months), birth length (n=25 at 6 months; 21 at 24 months), birthweight (n=8); HIV diagnosis timepoint (n=9), CD4 count (n=23), VL (n=92 at 6 months, n=86 at 24 months); ART initiation (n=5), ART regimen (n=8 at 6 months, n=7 at 24 months); infant prophylaxis (n=4 at 6 months, n=2 at 24 months).
All HIV-related variables are cited out of the number of HIV-infected mothers with available data. Percentages are cited among those with non-missing values.

a) Maternal smoking was measured by urine cotinine levels taken antenatally/birth urine using the IMMULITE® 1000 Nicotine Metabolite Kit (Siemens Medical Solutions Diagnostics®, Glyn Rhonwy, United Kingdom). Levels ≥500 ng/ml quantified active smoking, 10-500 mg/ml as passive smoking and <10ng/ml as non-smoking.
b) Maternal alcohol use assessed and quantified using the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) and retrospectively collected data on moderate-severe alcohol use in pregnancy forming a dichotomous measure.
c) Maternal depression in pregnancy measured using the Edinburgh postnatal depression scale (EPDS), a threshold of >=13 was used as the threshold for depression.
d) The lowest maternal CD4 within one year prior to birth and 3 months post-birth was used to reflect maternal immunosuppression in pregnancy and maximise sample numbers.
e) Maternal viral load determined as the highest viral load during pregnancy Where there was more than one result, the highest viral load during pregnancy was taken and <40 copies/ml was classified as lower than the detectable limit, >=40-<1000copies/ml as detectable and >=1000copies/ml as unsuppressed.

Appendix 3: Mean BSID-III scaled scores by domain according to HIV exposure at 6 and 24 months

BSID-III Domain: Scaled scores	6 months			24 months		
	Mean (SD)		P	Mean (SD)		P
	HIV-exposed uninfected	HIV-unexposed		HIV-exposed uninfected	HIV-unexposed	
Cognitive	10·73 (2·46)	10·18 (2·59)	0·14	6·80 (1·88)	7·14 (1·84)	0·039*
Receptive language	10·38 (2·75)	9·96 (2·78)	0·31	6·62 (1·82)	7·25 (1·97)	0·0002*
Expressive language	10·95 (3·56)	11·18 (3·33)	0·65	6·94 (2·29)	7·57 (2·30)	0·0023*
Fine Motor	13·15 (2·95)	12·76 (3·18)	0·40	9·25 (2·62)	9·29 (2·45)	0·83
Gross Motor	11·00 (2·59)	10·61 (2·93)	0·35	8·14 (2·15)	8·34 (2·40)	0·34

Footnote:

*P<0·05. Significance values are from unpaired t-tests. Scaled scores are standardised to a reference population and have a mean of 10 and SD of 3. Scores at 6 months are corrected for prematurity.

At 6 months, the mean scaled scores of all subscales were within the BSID-III reference range (mean 10, SD 3). At 24 months, HEU children had mean scaled scores <-1 SD below the reference mean in cognitive, receptive language and expressive language but not in fine or gross motor.

Appendix 4: Child developmental outcomes at 24 months according to HIV exposure assessing the effect of breastfeeding on the exposure-outcome relationship.

We assessed duration of exclusive breastfeeding and exclusive breastfeeding to 6 months (defined as the proportion of infants aged over 5 months fed exclusively with breastmilk). These breastfeeding variables were separately included in the adjusted models to examine any impact on the association between maternal HIV and child development. The adjusted differences and odds ratios in the table relate to the child developmental outcomes according to HIV exposure.

Raw scores						
BSID-III Domain	**Original multivariable model**		**Adjusted for exclusive breastfeeding duration**		**Adjusted for exclusive breastfeeding to 6 months**	
	Adjusted Difference (95% CI)**	**P**	**Adjusted difference (95% CI)****	**P**	**Adjusted difference (95% CI)****	**P**
Cognitive	-0·45 (-1·32 to 0·43)	0·32	-0·46 (-1·35 to 0·43)	0·32	-0·45 (-1·33 to 0·42)	0·31
Receptive language	-1·03 (-1·69 to -0·37)	0·0024*	-0·92 (-1·60 to -0·24)	0·0077*	-1·00 (-1·67 to -0·34)	0·0032*
Expressive language	-1·17 (-2·09 to -0·24)	0·013*	-1·10 (-2·05 to -0·15)	0·023*	-1·16 (-2·09 to -0·23)	0·015*
Fine Motor	0·09 (-0·49 to 0·66)	0·77	0·06 (-0·52 to 0·65)	0·83	0·07 (-0·50 to 0·65)	0·80
Gross Motor	-0·41(-1·09 to 0·27)	0·24	-0·46 (-1·15 to 0·23)	0·19	-0·40 (-1·09 to 0·28)	0·25
Delayed development (<-2SD)						
BSID-III Domain	**Original multivariable model**		**Adjusted for exclusive breastfeeding duration**		**Adjusted for exclusive breastfeeding to 6 months**	
	Adjusted Odds Ratio (95% CI)**	**P**	**Adjusted Odds Ratio (95% CI)****	**P**	**Adjusted Odds Ratio (95% CI)****	**P**
Cognitive	1·01 (0·55 to 1·85)	0·97	0·93 (0·50 to 1·74)	0·82	1·00 (0·55 to 1·84)	0·99
Receptive language	1·96 (1·09 to 3·52)	0·025*	1·94 (1·07 to 3·54)	0·030*	2·00 (1·11 to 3·60)	0·021*
Expressive language	2·14 (1·11 to 4·15)	0·024*	1·95 (0·99 to 3·86)	0·055	2·15 (1·11 to 4·17)	0·024*
Fine Motor	1·53 (0·53 to 4·42)	0·44	1·53 (0·52 to 4·53)	0·44	1·52 (0·52 to 4·43)	0·44
Gross Motor	1·23 (0·44 to 3·43)	0·69	1·18 (0·41 to 3·35)	0·76	1·23 (0·44 to 3·42)	0·70

Footnotes:

*p<0·05; For the linear regression models negative estimates indicate maternal HIV status is associated with lower total raw scores in that domain, and therefore poorer outcomes.
For the logistic regression models odds ratios >1 indicate maternal HIV status is associated with higher risk of delay in that domain, and therefore poorer outcomes.
**Adjusting for child age and child sex, maternal education, household income, maternal age and breastfeeding variables where indicated.

Appendix 5: Child developmental outcomes at 24 months according to HIV exposure assessing the effect of prematurity (<37 weeks) on the exposure-outcome relationship.

BSID-III Domain	Raw Scores		Delay (<-2SD)	
	Adjusted difference (95% CI)**	P	Adjusted Odds ratio (95% CI)**	P
Cognitive	-0·46 (-1·33 to 0·40)	0·29	1·02 (0·56 to 1·88)	0·94
Receptive language	-1·04 (-1·70 to -0·38)	0·0021*	1·96 (1·09 to 3·53)	0·024*
Expressive language	-1·18 (-2·10 to -0·25)	0·013*	2·16 (1·11 to 4·20)	0·023*
Fine Motor	0·08 (-0·49 to 0·65)	0·78	1·53 (0·53 to 4·43)	0·43
Gross Motor	-0·41 (-1·08 to 0·27)	0·24	1·21 (0·44 to 3·39)	0·71

Footnotes:

Similar results are obtained if low birth weight [<2·5kg] used in place of prematurity.

*p<0·05; For the linear regression models negative estimates indicate HIV exposure is associated with lower total raw scores in that domain, and therefore poorer outcomes. For the logistic regression models odds ratios >1 indicate maternal HIV status is associated with higher risk of delay in that domain, and therefore poorer outcomes.

**Adjusting for child age and child sex, maternal education, household income, maternal age and prematurity

Appendix 6: Child developmental outcomes at 24 months according to HIV exposure assessing the effect of maternal depression on the exposure-outcome relationship.

BSID-III Domain	Raw Scores		Delay (<-2SD)	
	Adjusted difference (95% CI)**	P	Adjusted Odds ratio (95% CI)**	P
Cognitive	-0·54 (-1·47 to 0·39)	0·26	1·04 (0·54 to 1·98)	0·91
Receptive language	-0·96 (-1·67 to -0·25)	0·0084*	1·78 (0·96 to 3·31)	0·066
Expressive language	-1·20 (-2·18 to -0·22)	0·016*	2·06 (1·01 to 4·23)	0·048*
Fine Motor	-0·02 (-0·64 to 0·59)	0·94	1·56 (0·49 to 4·93)	0·45
Gross Motor	-0·56 (-1·30 to 0·18)	0·14	1·27 (0·45 to 3·63)	0·65

Footnotes:

Antenatal maternal depression was measured using the Edinburgh Postnatal Depression Scale (EPDS) where a cut-off score of >=13 was used as the threshold for depression. Depression was used as a proxy for maternal psychosocial illness as per the DAG, however, further work needs to be done to assess this potential mediator.

*p<0·05; For the linear regression models negative estimates indicate HIV exposure is associated with lower total raw scores in that domain, and therefore poorer outcomes. For the logistic regression models odds ratios >1 indicate maternal HIV status is associated with higher risk of delay in that domain, and therefore poorer outcomes.

**Adjusting for child age and child sex, maternal education, household income, maternal age and maternal depression as measured by the Edinburgh Postnatal Depression Scale.

Appendix 7: Restricted analysis of the site with the majority of HIV-exposed uninfected children, where isiXhosa was the home language (n=388, HIV-exposed uninfected 155, HIV-unexposed 233). Unadjusted and adjusted BSID-III domain scores at 24 months according to HIV exposure.

Raw scores						
BSID-III Domain	**Mean (SD)**		**Unadjusted Difference (95% CI)**	**P**	**Adjusted Difference (95% CI)****	**P**
	HIV-exposed uninfected	**HIV-unexposed**				
Cognitive	54·77 (5·04)	55·55 (4·79)	-0·79 (-1·79 to 0·21)	0·12	-0·43 (-1·51 to 0·65)	0·43
Receptive language	19·79 (3·53)	20·86 (3·47)	-1·07 (-1·79 to -0·36)	0·0034*	-0·81 (-1·57 to -0·05)	0·037*
Expressive language	22·79 (5·39)	23·95 (4·60)	-1·16 (-2·19 to -0·13)	0·028*	-0·98 (-2·09 to 0·13)	0·082
Fine Motor	37·32 (3·33)	37·48 (3·22)	-0·16 (-0·83 to 0·51)	0·63	0·13 (-0·59 to 0·86)	0·72
Gross Motor	53·07 (3·41)	53·02 (4·03)	0·05 (-0·74 to 0·85)	0·90	-0·16 (-1·02 to 0·70)	0·71
Delayed development (<-2SD)						
BSID-III Domain	**N (%) with delay**		**Unadjusted OR (95% CI)**	**P**	**Adjusted OR (95% CI)****	**P**
	HIV-exposed uninfected	**HIV-unexposed**				
Cognitive	17 (11%)	22 (10%)	1·17 (0·60 to 2·28)	0·65	1·0 (0·48 to 2·07)	0.99
Receptive language	21 (14%)	15 (7%)	2·27 (1·13 to 4·56)	0·021*	1·96 (0·92 to 4·16)	0·081
Expressive language	17 (12%)	15 (7%)	1·84 (0·89 to 3·82)	0·099	2·35 (1·04 to 5·32)	0·041*
Fine motor	6 (4%)	7 (3%)	1·30 (0·43 to 3·94)	0·65	1·11 (0·33 to 3·77)	0·86
Gross motor	6 (4%)	11 (5%)	0·81 (0·29 to 2·25)	0·69	0·98 (0·31 to 3·14)	0·97

Footnotes:

The Afrikaans-speaking site had lower HEU numbers and therefore limited power to detect a difference.

*p<0·05; For the linear regression models negative estimates indicate HIV exposure is associated with lower total raw scores in that domain, and therefore poorer outcomes. For the logistic regression models odds ratios >1 indicate maternal HIV status is associated with higher risk of delay in that domain, and therefore poorer outcomes.

**Adjusting for child age and child sex, maternal education, household income and maternal age.

Appendix 8: Analysis to assess the potential effect of alcohol exposure in pregnancy on the exposure-outcome relationship.

This analysis was performed due to the difference seen between those children with and without a BSID-III at 24 months, as more children with a BSID-III performed were exposed to alcohol in pregnancy than those without.

BSID-III Domain	Raw Scores		Delay (<-2SD)	
	Adjusted Difference (95% CI)**	P	Adjusted Odds ratio (95% CI)**	P
Cognitive	-0·53 (-1·46 to 0·41)	0·27	1·08 (0·57 to 2·07)	0·81
Receptive language	-0·95 (-1·67 to -0·24)	0·0093*	1·85 (1·00 to 3·44)	0·052
Expressive language	-1·24 (-2·22 to -0·25)	0·014*	2·17 (1·06 to 4·48)	0·035*
Fine Motor	-0·03 (-0·65 to 0·59)	0·92	1·50 (0·47 to 4·78)	0·50
Gross Motor	-0·57 (-1·31 to 0·18)	0·13	1·28 (0·46 to 3·61)	0·64

Footnotes:

The same result was seen if the analysis was stratified by alcohol exposure.

*p<0·05; For the linear regression models negative estimates indicate HIV exposure is associated with lower total raw scores in that domain, and therefore poorer outcomes. For the logistic regression models odds ratios >1 indicate maternal HIV status is associated with higher risk of delay in that domain, and therefore poorer outcomes.

**Adjusting for child age and child sex, maternal education, household income, maternal age and alcohol exposure in pregnancy.

Appendix 9: Restricted analysis of those HIV-exposed uninfected children born to mothers on first line ART (n=132). Unadjusted and adjusted BSID-III domain scores according to HIV exposure.

Raw scores						
BSID-III Domain	**Mean (SD)**		**Unadjusted Difference (95% CI)**	**P**	**Adjusted Difference (95% CI)****	**P**
	HIV-exposed uninfected	**HIV-unexposed**				
Cognitive	55·18 (5·05)	55·69 (4·73)	-0·51 (-1·42 to 0·40)	0·27	-0·19 (-1·15 to 0·77)	0·70
Receptive language	20·00 (3·46)	21·10 (3·72)	-1·10 (-1·80 to -0·40)	0·0022*	-0·88 (-1·61 to -0·15)	0·019*
Expressive language	22·97 (5·58)	24·45 (4·94)	-1·48 (-2·47 to -0·49)	0·0035*	-1·12 (-2·15 to -0·10)	0·032*
Fine Motor	37·50 (3·28)	37·51 (3·10)	-0·01 (-0·61 to 0·58)	0·96	0·16 (-0·47 to 0·79)	0·61
Gross Motor	53·34 (3·22)	53·31 (3·66)	0·03 (-0·67 to 0·73)	0·94	-0·16 (-0·91 to 0·59)	0·68
Delayed development (< -2SD)						
BSID-III Domain	**N (%) with delay**		**Unadjusted OR (95% CI)**	**P**	**Adjusted OR (95% CI)****	**P**
	HIV-exposed uninfected	**HIV-unexposed**				
Cognitive	13 (10%)	52 (9%)	1·08 (0·57 to 2·05)	0·81	0·98 (0·49 to 1·96)	0·96
Receptive language	17 (13%)	40 (7%)	1·94 (1·06 to 3·55)	0·031*	1·93 (1·00 to 3·72)	0·049*
Expressive language	14 (11%)	31 (6%)	2·10 (1·08 to 4·07)	0·029*	2·41 (1·16 to 5·03)	0·019*
Fine motor	5 (4%)	12 (2%)	1·82 (0·63 to 5·26)	0·27	1·67 (0·53 to 5·25)	0·38
Gross motor	3 (2%)	19 (4%)	0·68 (0·20 to 2·33)	0·54	0·79 (0·21 to 3·00)	0·72

Footnotes:

*p<0·05; For the linear regression models negative estimates indicate maternal HIV status is associated with lower total raw scores in that domain, and therefore poorer outcomes. For the logistic regression models odds ratios >1 indicate maternal HIV status is associated with higher risk of delay in that domain, and therefore poorer outcomes.

**Adjusting for child age and child sex, maternal education, household income and maternal age at delivery.

Appendix VI: Supplementary Information Chapter 6

Early Neurodevelopment of HIV-Exposed Uninfected Children in the Era of Antiretroviral Therapy: a Systematic Review and Meta-analysis *(review paper)*

THE LANCET
Child & Adolescent Health

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Wedderburn CJ, Weldon E, Bertran-Cobo C, et al. Early neurodevelopment of HIV-exposed uninfected children in the era of antiretroviral therapy: a systematic review and meta-analysis. *Lancet Child Adolesc Health* 2022; published online April 25. https://doi.org/10.1016/ S2352-4642(22)00071-2.

Supplementary Information

Early neurodevelopment of HIV-exposed uninfected children in the era of antiretroviral therapy: a systematic review and meta-analysis

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[¶] AJP and KAD are joint senior authors

This PDF file includes:

S1 Appendix: PROSPERO systematic review protocol

Link: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42018075910

Protocol deviations:

- Studies including children born before January 2000 or where ART was documented not to be available at the time of the study were excluded. This differed to the original inclusion period of January 1990 for the date of search. We made this change in order to ensure relevance to the current ART era, fitting with the WHO guidelines advocating for PMTCT interventions in the year 2000.
- We were unable to assess vision, hearing, and intellect as outcomes due to a lack of appropriate studies within this age range.
- We used the validated National Heart, Lung, and Blood Institute (NHLBI) Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies (National Heart, Lung, and Blood Institute [NHLBI], 2014) to assess risk of bias instead of the originally specified Newcastle-Ottawa Quality Assessment Scale (NOS). We found this tool had many similarities to the NOS but allowed us to better assess the various aspects of bias for all types of studies included in this review.
- We performed formal risk of bias assessment for all Aim 1 outcomes which went forward into meta-analysis. Following review of the papers for Aim 2, we noted we were unable to perform a meta-analysis for the ART analyses due to study heterogeneity and that the risk of bias tool did not adequately focus on additional areas of concern specific to ART analyses. Therefore, instead of performing individual study risk of bias using a formal tool for these papers we describe the overall limitations and risk of bias specific to studies in Aim 2.
- Pre-specified sensitivity analyses included only studies with adequate comparison groups and excluding those with a moderate or high risk of bias. Due to limited numbers of studies fitting these criteria we did not perform subgroup analyses on breastfeeding. Similarly, we originally planned to stratify by region and socioeconomic status. However, given the limited number of studies, and lack of comparable reporting on socioeconomic status, we were only able to perform a sensitivity analysis of the meta-analysis excluding the one study from a high-income country.

S2 Appendix: Electronic search strategy by database

MEDLINE

1. (infan* or newborn* or new-born* or perinat* or neonat* or baby or babies or toddler* or child* or pediatric* or paediatric*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
2. exp CHILD, PRESCHOOL/
3. exp INFANT/
4. exp Pediatrics/
5. exp INFANT, NEWBORN/
6. 1 or 2 or 3 or 4 or 5
7. (HIV or HIV-1 or HIV-2 or human immunodeficiency virus or human immune deficiency virus or human immune-deficiency virus or HIV infect* or HIV-infected or living with HIV or HIV-positive or AIDS or Acquired Immunodeficiency Syndrome or Acquired Immune Deficiency Syndrome or HEU or HIV-exposed or HIV-exposed-uninfected or HIV-uninfected or HAART or antiretroviral* or anti-retroviral* or anti-HIV agents or HIV treatment or PMTCT or prevention of mother-to-child transmission).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
8. exp HIV/ or exp HIV-2/ or exp HIV-1/ or exp HIV INFECTIONS/
9. exp HIV PROTEASE INHIBITORS/ or exp ANTI-HIV AGENTS/ or exp HIV INTEGRASE INHIBITORS/ or exp HIV FUSION INHIBITORS/
10. exp Reverse Transcriptase Inhibitors/
11. exp Anti-Retroviral Agents/ or exp ANTIRETROVIRAL THERAPY, HIGHLY ACTIVE/ or exp Acquired Immunodeficiency Syndrome/
12. 7 or 8 or 9 or 10 or 11
13. (Neurodevelop* or (develop* adj4 child) or neurocog* or cogniti* or communication or language or speech or verbal or motor or neuromotor or locomotor or neuroanatom* or (brain adj4 structure) or (brain adj4 microstructure) or (white adj4 matter) or head circumference or (developmental adj4 delay*) or (developmental adj4 disorder*) or (developmental adj4 disabilit*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
14. exp CHILD DEVELOPMENT DISORDERS, PERVASIVE/ or exp MUSCULOSKELETAL DEVELOPMENT/ or exp HUMAN DEVELOPMENT/ or exp LANGUAGE DEVELOPMENT DISORDERS/ or exp "MALFORMATIONS OF CORTICAL DEVELOPMENT"/ or exp "MALFORMATIONS OF CORTICAL DEVELOPMENT, GROUP II"/ or exp "GROWTH AND DEVELOPMENT"/ or exp "MALFORMATIONS OF CORTICAL DEVELOPMENT, GROUP I"/ or exp LANGUAGE DEVELOPMENT/ or exp "NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT (U.S.)"/ or exp CHILD DEVELOPMENT/
15. exp CHILD BEHAVIOR/ or exp CHILD BEHAVIOR DISORDERS/
16. exp DEVELOPMENTAL DISABILITIES/
17. exp NEUROCOGNITIVE DISORDERS/ or exp NEURODEVELOPMENTAL DISORDERS/ or exp COMMUNICATION DISORDERS/
18. exp CHILD LANGUAGE/
19. exp Prenatal Exposure Delayed Effects/
20. 13 or 14 or 15 or 16 or 17 or 18 or 19
21. 6 and 12 and 20

EMBASE search

1. (infan* or newborn* or new-born* or perinat* or neonat* or baby or babies or toddler* or child* or pediatric* or paediatric*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]
2. exp preschool child/
3. exp infant/
4. exp pediatrics/
5. exp newborn/
6. 1 or 2 or 3 or 4 or 5

7. (HIV or HIV-1 or HIV-2 or human immunodeficiency virus or human immune deficiency virus or human immune-deficiency virus or HIV infect* or HIV-infected or living with HIV or HIV-positive or AIDS or Acquired Immunodeficiency Syndrome or Acquired Immune Deficiency Syndrome or HEU or HIV-exposed or HIV-exposed-uninfected or HIV-uninfected or HAART or antiretroviral* or anti-retroviral* or anti-HIV agents or HIV treatment or PMTCT or prevention of mother-to-child transmission).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]
8. exp Human immunodeficiency virus/ or exp Human immunodeficiency virus 2/ or exp Human immunodeficiency virus 1/
9. exp Human immunodeficiency virus infection/ or exp integrase inhibitor/ or exp Human immunodeficiency virus fusion inhibitor/ or exp Human immunodeficiency virus proteinase inhibitor/ or exp anti human immunodeficiency virus agent/ or exp nonnucleoside reverse transcriptase inhibitor/
10. exp antiretrovirus agent/ or exp highly active antiretroviral therapy/ or exp acquired immune deficiency syndrome/ or exp antiretroviral therapy/
11. 7 or 8 or 9 or 10
12. (Neurodevelop* or (develop* adj4 child) or neurocog* or cogniti* or communication or language or speech or verbal or motor or neuromotor or locomotor or neuroanatom* or (brain adj4 structure) or (brain adj4 microstructure) or (white adj4 matter) or head circumference or (developmental adj4 delay*) or (developmental adj4 disorder*) or (developmental adj4 disabilit*)).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word]
13. exp MUSCULOSKELETAL DEVELOPMENT/ or exp developmental language disorder/ or exp LANGUAGE DEVELOPMENT/ or exp child behavior/ or exp behavior disorder/
14. exp cognition/ or exp DEVELOPMENTAL DISORDER/
15. exp child development/
16. 12 or 13 or 14 or 15
17. 6 and 11 and 16

Pubmed search

infan* or newborn* or new-born* or perinat* or neonat* or baby or babies or toddler* or child or children or pediatric* or paediatric*

HIV or HIV-1 or HIV-2 or human immunodeficiency virus or human immune deficiency virus or human immune-deficiency virus or HIV infect* or HIV-infected or "living with HIV" or HIV-positive or AIDS or "Acquired Immunodeficiency Syndrome" or HEU or HIV-exposed or HIV-exposed-uninfected or HIV-uninfected or HAART or antiretroviral* or anti-retroviral* or "anti-HIV agents" or "HIV treatment" or PMTCT or "prevention of mother-to-child transmission"

Neurodevelop* or "child develop*" or neurocog* or cogniti* or communication or language or speech or verbal or motor or neuromotor or locomotor or neuroanatom* or "brain structure" or "brain microstructure" or "white matter" or "head circumference" or "developmental delay*" or "developmental disorder*" or "developmental disability"

1. 1 and 2 and 3

PsycINFO

1. (infan* or newborn* or new-born* or perinat* or neonat* or baby or babies or toddler* or child* or pediatric* or paediatric*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
2. exp preschool students/
3. exp pediatrics/
4. 1 or 2 or 3
5. (HIV or HIV-1 or HIV-2 or human immunodeficiency virus or human immune deficiency virus or human immune-deficiency virus or HIV infect* or HIV-infected or living with HIV or HIV-positive or AIDS or Acquired Immunodeficiency Syndrome or Acquired Immune Deficiency Syndrome or HEU or HIV-exposed or HIV-exposed-uninfected or HIV-uninfected or HAART or antiretroviral* or anti-retroviral* or anti-HIV agents or HIV treatment or PMTCT or prevention of mother-to-child

transmission).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
6. exp hiv/ or exp aids/
7. exp antiviral drugs/
8. 5 or 6 or 7
9. (Neurodevelop* or (develop* adj4 child) or neurocog* or cogniti* or communication or language or speech or verbal or motor or neuromotor or locomotor or neuroanatom* or (brain adj4 structure) or (brain adj4 microstructure) or (white adj4 matter) or head circumference or (developmental adj4 delay*) or (developmental adj4 disorder*) or (developmental adj4 disabilit*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
10. exp childhood development/ or exp early childhood development/ or exp motor development/ or exp psychological development/ or exp psychomotor development
11. exp brain development/
12. exp neonatal development/
13. exp language development/ or exp cognitive development/ or exp language delay/ or exp language disorders/ or exp speech development/
14. exp behavior problems/ or exp behavior/ or exp behavior disorders/
15. exp developmental disabilities/ or exp delayed development/
16. exp neurodevelopmental disorders/
17. exp Infant Development/
18. 9 or 10 or 11 or 12 or 14 or 15 or 16 or 17
19. 4 and 8 and 18

Global Health

1. (infan* or newborn* or new-born* or perinat* or neonat* or baby or babies or toddler* or child* or pediatric* or paediatric*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
2. Exp PRESCHOOL CHILDREN/
3. Exp INFANTS/
4. exp PAEDIATRICS/
5. 1 or 2 or 3 or 4
6. (HIV or HIV-1 or HIV-2 or human immunodeficiency virus or human immune deficiency virus or human immune-deficiency virus or HIV infect* or HIV-infected or living with HIV or HIV-positive or AIDS or Acquired Immunodeficiency Syndrome or Acquired Immune Deficiency Syndrome or HEU or HIV-exposed or HIV-exposed-uninfected or HIV-uninfected or HAART or antiretroviral* or anti-retroviral* or anti-HIV agents or HIV treatment or PMTCT or prevention of mother-to-child transmission).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
7. exp acquired immune deficiency syndrome/
8. exp human immunodeficiency viruses/ or exp hiv infections/ or exp human immunodeficiency virus 1/ or exp human immunodeficiency virus 2/
9. exp hiv fusion inhibitors/ or exp antiretroviral agents/ or exp hiv integrase inhibitors/ or exp hiv protease inhibitors/
10. exp reverse transcriptase inhibitors/ or exp non-nucleoside reverse transcriptase inhibitors/ or exp nucleoside reverse transcriptase inhibitors/ or exp nucleotide reverse transcriptase inhibitors/
11. exp antiviral agents/
12. 6 or 7 or 8 or 9 or 10 or 11
13. (Neurodevelop* or (develop* adj4 child) or neurocog* or cogniti* or communication or language or speech or verbal or motor or neuromotor or locomotor or neuroanatom* or (brain adj4 structure) or (brain adj4 microstructure) or (white adj4 matter) or head circumference or (developmental adj4 delay*) or (developmental adj4 disorder*) or (developmental adj4 disabilit*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]
14. exp child development/
15. exp early childhood development/ or exp infant development/
16. exp pervasive child development disorders/

17. exp development/
18. exp cognitive development/ or exp psychomotor development/ or exp motor development/
19. exp speech development/
20. 13 or 14 or 15 or 16 or 17 or 18 or 19
21. 5 and 12 and 20

Africa-wide information
(infan* or newborn* or new-born* or perinat* or neonat* or baby or babies or toddler* or child* or pediatric* or paediatric*) AND (HIV or HIV-1 or HIV-2 or human immunodeficiency virus or human immune deficiency virus or human immune-deficiency virus or HIV infect* or HIV-infected or living with HIV or HIV-positive or AIDS or Acquired Immunodeficiency Syndrome or Acquired Immune Deficiency Syndrome or HEU or HIV-exposed or HIV-exposed-uninfected or HIV-uninfected or HAART or antiretroviral* or anti-retroviral* or anti-HIV agents or HIV treatment or PMTCT or prevention of mother-to-child transmission) AND (Neurodevelop* or (develop* N4 child) or neurocog* or cogniti* or communication or language or speech or verbal or motor or neuromotor or locomotor or neuroanatom* or (brain N4 structure) or (brain N4 microstructure) or (white N4 matter) or head circumference or (developmental N4 delay*) or (developmental N4 disorder*) or (developmental N4 disabilit*))

S3 Appendix: PECO inclusion and exclusion criteria

	Inclusion criteria	Exclusion criteria
Population	• Children aged 0 to 5 years • Born after January 2000 (WHO first issued recommendations for antiretroviral drugs in 2000[1])	• Studies of children >5 years that did not include younger children* • Children born before the year 2000
Exposure	• HIV exposure without HIV infection. HEU children were defined as children born to mothers with HIV infection with appropriate reporting confirming that children were not infected • ART exposure was defined as exposure to any maternal antiretroviral drugs during pregnancy	• No documentation of maternal and child HIV infection status • Children with HIV infection • Studies where ART was noted to be unavailable at the time of the study • Studies of HEU children where the primary scientific question related to other exposures and relevant data could not be extracted (e.g. nutritional deficiencies or other infections)
Control	• Children born to HIV-uninfected mothers (Aim 1) AND/OR • HEU children exposed to different maternal ART regimens, classes or drugs or no treatment (Aim 2)	• Studies without either of these control groups
Outcomes	***Primary***: • Cognitive development, receptive language, expressive language, fine motor, gross motor (as recommended for children 0-5 years corresponding to developmental areas in the ICD-10 and -11 Delayed Milestones definitions[2]) • Social-emotional and adaptive behaviour (defined as daily living skills that enable everyday function appropriate to the relevant age group[2]). • Measured using validated instruments. ***Secondary***: • Brain structure, as measured by neuroimaging • Head circumference	• Other outcomes
Study type	• All primary data study designs • Reviews and meta-analyses were hand-searched for additional primary studies • Academic publications • English or Spanish language	• Conference and poster abstracts • Languages other than English and Spanish

*Studies were included if they had stratified analyses of the population of interest and the majority of children fulfilled the inclusion criteria. If the age range extended through 5 years, studies were included if the median age was <5 years.

S4 Appendix: Quality assessment tool

NIH Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies criteria

1. Was the research question or objective in this paper clearly stated?
2. Was the study population clearly specified and defined?
3. Was the participation rate of eligible persons at least 50%?
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?
5. (5.a) Was a sample size justification, power description, or variance and effect estimates provided?
(5.b) Was sample size ≥ 50 per group?*
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)? For the purposes of this review we assessed whether studies examined results by maternal CD4, viral load or ART.**
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants? For the purposes of this review we assessed for valid HIV testing of mothers of HEU and HU children for ascertainment of exposure.***
10. Was the exposure(s) assessed more than once over time? For the purposes of this review we assessed valid HIV testing of HIV-exposed children and that children with HIV were excluded.***
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?
12. Were the outcome assessors blinded to the exposure status of participants?
13. Was loss to follow-up after baseline 20% or less
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?

Risk of bias rating: Yes; No; CD, cannot determine; NA, not applicable; NR, not reported

Overall Study Quality rating: Good, Fair, Poor

* Question five was specifically adapted for this review per Carbia *et al*, 2018;
**Question eight was adapted for this review to assess if studies examined results by maternal CD4/viral load/ART to lend credibility to the hypothesis of causality between exposure and outcome;
***Given the importance of accurately assessing HIV exposure and avoidance of child HIV infection we adapted questions nine and ten to assess whether mothers of both HEU and HU children received valid HIV testing and that children who were HIV-exposed received valid HIV testing.

S5 Appendix: Characteristics of studies excluded following full-text review

No.	Year	Author	Title	Journal	Exclusion criteria
1	2009	Abubakar, A. *et al*	The role of weight for age and disease stage in poor psychomotor outcome of HIV-infected children in Kilifi, Kenya	*Dev Med Child Neurol*	No ART exposure – ART not routinely available at the time of the study
2	2013	Abubakar, A. *et al*	The performance of children prenatally exposed to HIV on the A-not-B task in Kilifi, Kenya: A preliminary study	*Int J Environ Res Public Health*	No ART exposure – ART not routinely available at the time of the study
3	2014	Abubakar, A.	Biomedical risk, psychosocial influences, and developmental outcomes: lessons from the pediatric HIV population in Africa	*New directions for child and adolescent development*	Review article; no new data.
4	2016	Ackermann, C. *et al*	Early antiretroviral therapy in HIV-infected children is associated with diffuse white matter structural abnormality and corpus callosum sparing	*American Journal of Neuroradiology*	No HEU group (parallel vaccine study included HEU and HU children combined in this paper); Age >5 years (control group mean 5.7 years)
5	2020	Ackermann, C *et al*	Diffusion tensor imaging point to ongoing functional impairment in HIV-infected children at age 5, undetectable using standard neurodevelopmental assessments	*AIDS Research and Therapy*	No distinct HEU group (control group of 11 from parallel vaccine study which included 9 HEU and 2 HU combined in this paper); Age >5 years included (control group mean 5.6 years)
6	2019	Ajayi, OR. *et al*	Association of anthropometric status and residential locality factors with cognitive scores of 4-6-year-old children in Kwazulu-Natal, South Africa	*South African Journal of Clinical Nutrition*	No HEU group; Age >5 years (4-6 years) and no stratification
7	2016	Alcock, KJ. *Et al*	The effect of prenatal HIV exposure on language functioning in Kenyan children: establishing an evaluative framework	*BMC Res Notes*	No ART exposure – ART not routinely available at the time of the study
8	2013	Andronikou, S.	Corpus callosum thickness on MRI as a surrogate marker of brain volume in children with HIV and its correlation with developmental scores and clinical parameters	*Pediatric Radiology*	No HEU group; Conference presentation
9	2001	Bakaki, P. *et al*	Epidemiologic and clinical features of HIV-infected and HIV-uninfected Ugandan children younger than 18 months	*JAIDS*	No distinct HEU group - HEU and HU grouped together as 'HIV-uninfected'
10	2016	Bass, J.K. *et al*	Association of caregiver quality of care with neurocognitive outcomes in HIV-affected children aged 2-5 years in Uganda	*AIDS Care*	Cohort outcomes reported in Ruiseñor-Escudero *et al.*
11	2019	Blakstad, M.M. *et al*	Nutritional, socioeconomic, and delivery characteristics are associated with neurodevelopment in Tanzanian children	*Journal of Pediatrics*	HEU data reported in Manji KP *et al. (NCT00197730 trial)*
12	2001	Blanchette, N. *et al*	Cognitive and motor development in children with vertically transmitted HIV infection	*Brain and Cognition*	Cohort spanned years <2000
13	2016	Blokhuis, C. *et al*	The Eye as a Window to the Brain: Neuroretinal Thickness Is Associated With Microstructural White Matter Injury in HIV-Infected Children	*Investigative Ophthalmology & Visual Science*	No specified HEU group; Age >5 years (mean 12.1 years)
14	2017	Boateng, G.O. e*t al*	Early childhood learning activities buffer adverse effects of HIV exposure on infant cognitive development: A longitudinal study	*FASEB Journal*	No specified HEU group - 'HIV-exposed' group analysed which may include HIV-infected children; Poster presentation
15	2014	Boivin, M.J. *et al*	Bouts of malaria illness as mediated by anemia diminishes cognitive development in very young Ugandan children	*American Journal of Tropical Medicine and Hygiene*	Conference abstract (paper included in full reviews). No distinct HEU group – 'HIV-exposed' group analysed which may include HIV-infected children
16	2017	Boivin, M.J. *et al*	Effect of caregiver training on neurodevelopment of HIV-exposed uninfected children and caregiver mental health: a Ugandan cluster randomized controlled trial	*J Dev Behav Pediatr*	Intervention trial. ART exposure not mentioned
17	2018	Boivin, M.J. *et al*	Neuropsychological performance in African children with HIV enrolled in a multisite antiretroviral clinical trial	*AIDS*	Age >5 years (5-11 years)
18	2018	Boivin, M., *et al*	Developmental and cognitive effects of type of antepartum and postpartum ARV exposure for Ugandan and Malawian IMPACCT PROMISE HIV-exposed versus unexposed children at age 12, 24 and 48 months	*JIAS*	Conference presentation, paper included in full texts
19	2018	Boivin, M.J. *et al*	African multi-site 2-year study of neurocognition in HIV infected/affected children	*Topics in Antiviral Medicine (CROI 2018)*	Age >5 years (5-11 years)
20	2019	Boivin, M.J. *et al*	African multi-site 2-year neuropsychological study of school-age children perinatally infected, exposed, and unexposed to human immunodeficiency virus	*CID*	Age >5 years (5-11 years)
21	2020	Boivin, M.J. *et al*	Early Childhood Development Caregiver Training and Neurocognition of HIV-exposed Ugandan Siblings	*Journal of Developmental & Behavioral Pediatrics*	Age >5 years (5-12 years)
22	2008	Botha, J.A.E.	Motor development and growth status of 2 to 6 year old children infected with Human Immunodeficiency Virus [HIV]	*NA*	Thesis
23	2008	Botha, JAE & Pienaar AE.	The motor development of 2 to 6-year old children infected with HIV	*SA Journal for Research in Sport, Physical Education and Recreation*	No ART exposure – access to ART was limited at the time of the study
24	2011	Bowes, J. *et al*	Pervasive developmental disorder in antiretroviral- and HIV-exposed, uninfected children	*Canadian Journal of Infectious Diseases*	Cohort spanned years <2000 (1997-2010); Conference presentation

				and Medical Microbiology	
25	2009	Briand, N. *et al*	No relation between in-utero exposure to HAART and intrauterine growth retardation	*AIDS*	Cohort spanned years <2000 (children born 1990 – 2006); although stratified by year only n=23 have head circumference measured in the >2000 group, <10% of cohort
26	2001	Bruck, I. *et al*	Developmental milestones of vertically HIV infected and seroreverters children: follow up of 83 children	*Arquivos de neuro-psiquiatria*	Cohort spanned years <2000 (children born 1995 – 2000)
27	2018	Budd, M.A. *et al*	Blood mitochondrial DNA content in HIV-exposed uninfected children with autism spectrum disorder	*Viruses*	Age > 5 years (2-16 years)
28	2003	Bulgheroni, S. *et al*	Longitudinal neuropsychological evaluation of neurologically asymptomatic HIV infected children	*Psicologia Clinica dello Sviluppo*	Age > 5 years included (4 – 15 years); Italian
29	2015	Buonomo, E., *et al*	Malnutrition decreases the odds of attaining motor milestones in HIV exposed children: results from a paediatric DREAM cohort	*Epidemiologia e prevenzione*	HEU group included HIV-infected child
30	2013	Cambrea, S.C. *et al*	Can HIV infection during pregnancy cause an intrauterine growth restriction?	*BMC Infectious Diseases*	No distinct HEU group - HEU and HIV-infected children grouped together; Conference presentation
31	2014	Cambrea, S.C. *et al*	Evaluation of anthropometric and virologic data in newborn from HIV positive mothers	*BMC Infectious Diseases*	No distinct HEU group - HEU and HIV-infected children grouped together; Conference presentation
32	2009	Carcellar, A. *et al*	Lack of effect on prematurity, birth weight, and infant growth from exposure to protease inhibitors in utero and after birth	*Pharmaco-therapy*	Cohort spanned years <2000 (1997-2005)
33	2020	Chandna, J. *et al*	Effects of improved complementary feeding and improved water, sanitation and hygiene on early child development among HIV-exposed children: substudy of a cluster randomized trial in rural Zimbabwe	*BMJ Global Health*	Main study results presented in Ntozini, R *et al,* included
34	2000	Chase, C. *et al*	Early cognitive and motor development among infants born to women infected with human immunodeficiency virus.	*Pediatrics*	Cohort spanned years <2000 (children born 1990 onwards)
35	2011	Chaworth-Musters, T. *et al*	Adverse health outcomes in HIV exposed uninfected children (HEU) in British Columbia - CIHR team grant in HIV therapy and aging (CARMA)	*Canadian Journal of Infectious Diseases and Medical Microbiology*	Age >5 years included (mean 5.4 years, range 0.6-19.6 years); Conference presentation
36	2018	Chernoff, M. *et al*	Assessing Psychiatric Symptoms in Youth Affected by HIV: Comparing a Brief Self-Administered Rating Scale with a Structured Diagnostic Interview	*Journal of Clinical Psychology in Medical Settings*	Age >5 years (range 6-18 years)
37	2018	Chernoff, M.C. *et al*	Validity of neuropsychological testing in young African children affected by HIV	*Journal of Pediatric Infectious Diseases*	Age >5 years (range 5-11 years)
38	2018	Chhaya, R. *et al*	The feasibility of an automated eye-tracking-modified Fagan test of memory for human faces in younger Ugandan HIV-exposed children	*Child Neuropsychology*	Feasibility study for automated eye-tracking. No mention of ART
39	2005	Chiriboga, C.A. *et al*	Incidence and prevalence of HIV encephalopathy in children with HIV infection receiving highly active anti-retroviral therapy (HAART)	*Journal of Pediatrics*	No HEU group; Cohort spanned years <2000 (followed up from 1988 onwards)
40	2018	Chingono, R. *et al*	Evaluating the effectiveness of a multi-component intervention on early childhood development in paediatric HIV care and treatment programmes: a randomized controlled trial	*BMC Pediatrics*	Trial protocol, no results
41	2019	Christodoulou, J. *et al*	Perinatal maternal depression in rural South Africa: Child outcomes over the first two years	*Journal of Affective Disorders*	No HEU group or stratification by HIV status
42	2019	Cockcroft, K. and Milligan, R.	Working memory structure in atypical development: HIV-infected and HIV-exposed, uninfected school beginners	*Developmental Neuropsychology*	Age >5 years (HEU mean age 7.36 years, SD 0.88)
43	2017	Coelho, A.V. *et al*	Antiretroviral Treatment in HIV-1-Positive Mothers: Neurological Implications in Virus-Free Children	*International Journal of Molecular Sciences*	Review
44	2015	Conserve, D.G., *et al*	Maternal HIV illness and its impact on children well-being and development in Haiti	*J Child Fam Stud*	No neurodevelopment outcomes; focus on caregiver not children; no distinct HEU group
45	2018	Dalili, D. *et al*	Growth and development status in the first two years of uninfected children born from HIV positive mothers	*Acta Medica Iranica*	No HU control group or statistical comparison with normative data
46	2016	Das, P.K. *et al*	Abundance of psychiatric morbidity in perinatally HIV infected children and adolescents with comparison to their HIV negative sibling	*Neurology Psychiatry and Brain Research*	Age >5 years included (stratification at 6 years)
47	2016	Datong, P *et al*	Breast-fed HIV-1 exposed infants play catch up	*BMC Infectious Diseases Conference*	Conference abstract; No distinct HEU group – no statement HIV exposed children are all uninfected
48	2000	Davis-McFarland, E.	Language and oral-motor development and disorders in infants and young toddlers with human immunodeficiency virus	*Seminars in speech and language*	Review of HIV-infected children
49	2018	Debeaudrap, P. *et al*	Neurodevelopmental outcomes in HIV-infected and uninfected African children	*AIDS*	Age >5 years (4-9 years, mean HEU 6.2 years, HU 6.2 years)
50	2003	Del Pilar Kufa, M. *et al*	Neurodevelopment in HIV-exposed children	*Interdisciplinaria Revista de*	No distinct HEU group – infants born to HIV-infected women analysed which may include HIV-infected children

				Psicologia y Ciencias Afines	
51	2016	Desmonde, S.T. *et al*	Health and survival of HIV perinatally exposed but uninfected children born to HIV-infected mothers	*Current opinion in HIV and AIDS*	Review
52	2019	Donald, K.A. *et al*	Risk and protective factors for child development: An observational South African birth cohort	*PLOS Medicine*	HEU outcomes reported in *Wedderburn, C.J. et al*
53	2019	Ekali, G.L. *et al*	Effect of in utero exposure to HIV and antiretroviral drugs on growth in HIV-exposed uninfected children: a systematic review and meta-analysis protocol	*BMJ Open*	Review; protocol without results
54	2016	Evans, C.V. *et al*	Head circumferences of children born to HIV-infected and HIV-uninfected mothers in Zimbabwe during the preantiretroviral therapy era	*AIDS*	Cohort spanned years <2000 (1997-2000)
55	2016	Ezeamama, A.E. *et al*	Perinatal HIV Status and Executive Function During School-Age and Adolescence: A Comparative Study of Long-Term Cognitive Capacity Among Children From a High HIV Prevalence Setting	*Medicine*	Age >5 years (cognitive assessment between 6-18 years, school age)
56	2019	Ezeamama, A.E. *et al*	Serum vitamin D is differentially associated with socioemotional adjustment in early school-aged Ugandan children according to perinatal HIV status and in utero or peripartum antiretroviral exposure history	*American Journal of Tropical Medicine and Hygiene*	Age >5 years (6-10 years, mean HEU 7.5 years, HUU 7.6 years); conference presentation (full paper checked: Yakah, W. *et al* Nutrients 2019;11(7):1570)
57	2020	Familiar, I. *et al*	Association between caregiver depression symptoms and child executive functioning. Results from an observational study carried out in four sub-Saharan countries	*AIDS Care*	Age >5 years (5-11 years; HEU mean 7.3 years, HUU 7.3 years)
58	2014	Fasunla, A.J.O.G. *et al*	Comparison of hearing status of HIV-exposed and-unexposed newborns and immunovirologic correlates in HIV-exposed newborns	*Otolaryngology - Head and Neck Surgery (United States)*	Conference abstract; No distinct HEU group - 'HIV-exposed' group analysed which may include HIV-infected children
59	2014	Fasunla A.J. *et al*	Comparison of auditory brainstem response in HIV-1 exposed and unexposed newborns and their correlation with the maternal viral load and CD4 cell counts	*AIDS*	Paper from above; HEU group not clearly defined; Outcome not included.
60	2009	Ferguson, G. *et al*	The prevalence of motor delay among HIV infected children living in Cape Town, South Africa	*International Journal of Rehabilitation Research*	No specified HEU group – reference sample unknown HIV-status
61	2011	Ferguson, G. *et al*	The motor development of orphaned children with and without HIV: Pilot exploration of foster care and residential placement	*Physiotherapy (United Kingdom)*	Conference abstract; No HEU group
62	2013	Ferguson, K.T. *et al*	Cognitive, motor, and behavioral development of orphans of HIV/AIDS in institutional contexts	*Neuropsychology of children in Africa: Perspectives on risk and resilience*	No distinct HEU group (20% of children had been diagnosed with HIV/AIDS)
63	2009	Fernández Ibieta, M. *et al*	Growth of uninfected infants exposed to antiretrovirals born to HIV-infected women	*Anales de Pediatria*	No control group
64	2000	Fishkin, P.E. *et al*	Brief report: relationship between HIV infection and WPPSI-R performance in preschool-age children	*Journal of Pediatric Psychology*	No HEU group; Cohort spanned years <2000 (1999+)
65	2018	Fouché, C. *et al*	Anthropometric parameters of HIV-infected and HIV-uninfected mothers and their premature infants	*Journal of Tropical Pediatrics*	No distinct HEU group - 'HIV-infected mothers and their premature infants analysed which may include HIV-infected children
66	2019	Gaulee Pokhrel, K. V. D. *et al*	Influence of stigma and discrimination on psychosocial health in children affected by AIDS in Nepal: A cross-sectional study	*HIV Medicine*	No distinct HEU group; Age > 5 years (2-14 years); Conference abstract
67	2020	Ge, X.M. *et al*	Influence on physical development of children aged 18 months from HIV-positive mothers for prevention mother to child transmission of HIV	*Zhonghua liu xing bing xue za zhi*	Anthropometry measures grouped, no head circumference. Language of full-text: Chinese
68	2017	Gonzalez, R. *et al*	Effects of HIV infection on maternal and neonatal health in southern Mozambique: a prospective cohort study after a decade of antiretroviral drugs roll out	*PLoS ONE*	No distinct HEU group - infants born to HIV-infected women which may include HIV-infected children
69	2012	Gompels, U.A *et al*	Human Cytomegalovirus Infant Infection Adversely Affects Growth and Development in Maternally HIV-Exposed and Unexposed Infants in Zambia	*CID*	No distinct HEU group - separated by maternal HIV status but not combined with child status so HIV-infected children included with HEU group
70	2011	Griner, R. *et al*	In utero and postnatal exposure to antiretrovirals among HIV-exposed but uninfected children in the United States	*AIDS Patient Care STDS*	Cohort spanned years <2000 (1995 to 2009); No neurodevelopmental outcomes
71	2008	Grosch-Woerner, I. *et al*	Increased rate of prematurity associated with antenatal antiretroviral therapy in a German/Austrian cohort of HIV-1-infected women	HIV Medicine	Cohort spanned years <2000 (1995-2001)
72	2005	Hankin, C. *et al*	Does exposure to antiretroviral therapy affect growth in the first 18 months of life in uninfected children born to HIV-infected women?	*JAIDS*	Cohort spanned years <2000 (1985+)
73	2018	Heffron, R. *et al*	Pregnancy outcomes and infant growth among babies with in-utero exposure to tenofovir-based preexposure prophylaxis for HIV prevention	*AIDS*	No HEU children
74	2016	Hermetet-Lindsay, K.D.	Contributions of disease severity, psychosocial factors, and cognition to behavioral functioning in youth perinatally exposed to HIV	*Dissertation Abstracts*	Age includes >5 years (school age: mean 10.9 years); Dissertation

75	2013	Herrero, D. *et al*	Motor development of infants exposed to maternal human immunodeficiency virus (HIV) but not infected	*International Archives of Medicine*	No HU control group or statistical comparison with normative data.
76	2015	Himes, S.K. *et al*	Meconium atazanavir concentrations and early language outcomes in HIV-exposed, uninfected infants with prenatal atazanavir exposure	*JAIDS*	No control group. Focus on meconium concentrations
77	2008	Hochhauser, C.J. *et al*	The impact of environmental risk factors on HIV-associated cognitive decline in children	*AIDS Care*	No HEU group; Cohort spanned years <2000 (1993-onwards); Age >5 years (1-13 years)
78	2001	Holditch-Davis, D. *et l*	Parental caregiving and developmental outcomes of infants of mothers with HIV	*Nursing research*	Cohort spanned years <2000 (children born before year 2000).No distinct HEU group
79	2011	Hutchings, J.	Developmental delay in HIV-exposed infants attending Newlands Clinic in Harare, Zimbabwe	*NA*	Dissertation
80	2014	Hutchings, J. & Potterton, J.	Developmental delay in HIV-exposed infants in Harare, Zimbabwe	*Vulnerable children and youth studies*	No HU control group or statistical comparison with normative data.
81	2019	Iloghalu, E.I. *et al*	Effect of maternal HIV infection on treatment with HAART on neonatal birth weight and other anthropometry: A cohort study of HIV sero-positive women in Enugu, South-East Nigeria	*Journal of Clinical and Diagnostic Research*	No distinct HEU group - infants born to HIV sero-positive women which may include HIV-infected children
82	2017	Iloh, K.K. *et al*	Neurocognitive function of school-aged HIV-infected children in Enugu, Nigeria	*Journal of Tropical Pediatrics*	No specified HEU group; Age>5 years (6-15 years)
83	2018	Jacobson, D. *et al*	Alcohol use among HIV-infected pregnant women and child outcomes in the Pediatric HIV AIDS Cohort study (PHACS)	*Alcoholism: Clinical and Experimental Research*	Conference presentation; substance use confounder; age range for neurodevelopment evaluations not clear (children followed up until age 18)
84	2017	Jankiewicz, M. *et al*	White matter abnormalities in children with HIV infection and exposure	*Frontiers in Neuroanatomy*	Age (follow up >5 years; mean age 7.3 years)
85	2020	Jantarabenjakul W. *et al*	Behavioral problems in perinatally HIV-infected young children with early antiretroviral therapy and HIV-exposed uninfected young children: prevalence and associated factors	*AIDS Care*	Neurodevelopmental outcomes reported in other paper (*Jantarabenjakul W. et al., 2019)*
86	2019	Jantarabenjakul W. *et al*	Low risk of neurodevelopmental impairment among perinatally acquired HIV-infected preschool children who received early antiretroviral treatment in Thailand	*JIAS*	No HU control group or statistical comparison with normative data
87	2015	Jao, J. *et al*	Growth patterns in the first year of life differ in infants born to perinatally vs. non-perinatally HIV-infected women	*AIDS*	No neurodevelopmental outcomes or OFC
88	2020	Jao, J. *et al*	Neurodevelopment of HIV-exposed uninfected infants born to women with perinatally acquired HIV in the United States	*J Acquir Immune Defic Syndr*	Comparison of perinatally versus non-perinatally acquired HIV in mothers. SMARTT cohort and neurodevelopmental outcomes reported in included papers
89	2011	Jelsma, J. *et al*	The motor development of orphaned children with and without HIV: Pilot exploration of foster care and residential placement	*BMC Pediatrics*	No distinct HEU group (children infected with HIV compared with those without HIV residing in institutions or with foster parents, maternal HIV status not documented)
90	2016	Kaaya, S. *et al*	Association of maternal depression and infant nutritional status among women living with HIV in Tanzania	*Maternal & child nutrition*	No neurodevelopmental outcomes; 20% infants of unknown HIV status; cohort spanned years <2000 (1995 – 1997)
91	2016	Kakkar, F.W. *et al*	Safety of combination antiretroviral prophylaxis in high-risk HIV-exposed newborns: A retrospective review of the Canadian experience	*JIAS*	Cohort spanned years <2000 (1997-2013); No distinct HEU group - 'high-risk HIV-exposed' group analysed which may include HIV-infected children
92	2012	Kapetanovic, S. *et al*	T-cell activation and neurodevelopmental outcomes in perinatally HIV-infected children	*AIDS*	No HEU group
93	2004	Keller, M.A. *et al*	Altered neurometabolite development in HIV-infected children: Correlation with neuropsychological tests	*Neurology*	No specified HEU group; Age >5 years (range 6-16 years)
94	2014	Kerr, S.J. *et al*	Neurodevelopmental outcomes in HIV-exposed-uninfected children versus those not exposed to HIV	*AIDS Care*	Age includes >5 years and no subanalysis (1-12 years, mean 7.6 years
95	2000	Knight, W.G. *et al*	Brief report: Effects of pediatric HIV infection on mental and psychomotor development	*Journal of Pediatric Psychology*	Cohort spanned years <2000
96	2018	Knox, J. *et al*	Screening for developmental disabilities in HIV positive and HIV negative children in South Africa: Results from the Asenze Study	*PLoS One*	No distinct HEU group - separated by child HIV status but not the combined HEU v. HUU groupings – 'HIV-negative group contains HEU and HU children
97	2004	Kullgren, K.A. *et al*	Prediction of cognitive, adaptive, and behavioral functioning in preschool and school-age children with HIV	*Children's Health Care*	No HEU group; Age > 5 years (3 to 16 years and no stratification)
98	2014	Kuona, P. *et al*	Growth and development of the HIV exposed uninfected children below 5 years in developing countries: focus on nutritional challenges, mortality and neurocognitive function. (Special issue on malnutrition.)	*Food and Nutrition Sciences*	Review
99	2000	Layton, T. *et al*	Language development and assessment in children with human immunodeficiency virus: 3 to 6 years	*Seminars in speech and language*	Review
100	2012	Le Doare, K. *et al*	Neurodevelopment in children born to HIV-infected mothers by infection and treatment status	*Pediatrics*	Review
101	2018	Le Roux, S.M. *et al*	HIV viraemia in pregnancy and neurodevelopment of HIV-exposed uninfected children	*Topics in Antiviral Medicine*	Conference presentation

102	2018	Le Roux, S.M. *et al*	HIV viremia during pregnancy and neurodevelopment of HIV-exposed uninfected children in the context of universal antiretroviral therapy and breastfeeding	*HIV Reports*	Neurodevelopmental outcomes reported in separate paper Le Roux et al 2018, included in analysis
103	2019	Le Roux, K. *et al*	A longitudinal cohort study of rural adolescent vs adult South African mothers and their children from birth to 24 months	*BMC Pregnancy and Childbirth*	No HEU group - only HIV status of mother described
104	2009	Li, Y. *et al*	A study on clinical characteristics, growth development, and intelligence of child patients with Human Immunodeficiency Virus type	*Chinese Journal of Clinical Psychology*	Language of full-text: Chinese; No distinct HEU group - 'HIV-infected versus normal children'
105	2016	Li, Y.X. *et al*	Physical development of HIV-exposed infants in Kunming city: a cohort study	*Maternal and Child Health Care of China*	Language of full-text: Chinese; No developmental outcomes
106	2009	Lin, XY. *et al*	Physical and psychological health among children affected by HIV/AIDS: Difference in groups and caring arrangements	*Chinese Journal of Clinical Psychology*	Language of full-text: Chinese; No distinct HEU group - 'Participants were double AIDS orphans, single AIDS orphans and affected children who were taken care of by family and orphanage'
107	2007	Lindsey, J.C. *et al*	Neurodevelopmental functioning in HIV-infected infants and young children before and after the introduction of protease inhibitor-based highly active antiretroviral therapy	*Pediatrics*	Cohort spanned years <2000 (1993-onwards; cohort demographics grouped by year of birth but not development)
108	2015	Linn, K. *et al*	HIV-Related Cognitive Impairment of Orphans in Myanmar with Vertically Transmitted HIV Taking Antiretroviral Therapy	*Pediatric Neurology*	No distinct HEU group - 'HIV-infected versus HIV-negative children in orphanages' No further information on maternal HIV status of HIV-negative children
109	2016	Liotta, G. *et al*	Growth indices in breastfed infants pre and postnatally exposed to tenofovir compared with tenofovir-unexposed infants	*AIDS*	No neurodevelopmental outcomes
110	2002	Lipman, T.H. *et al*	Assessment of growth and immunologic function in HIV-infected and exposed children	*Journal of the Association of Nurses in AIDS Care*	No neurodevelopmental outcomes; Includes children >5 years (0-14 years)
111	2016	Louw, K.A. *et al*	Correlates of emotional and behavioural problems in children with perinatally acquired HIV in Cape Town, South Africa	*AIDS Care*	No HEU group; Age includes >5 years (6-16 years)
112	2001	Macmillan, C. *et al*	Head growth and neurodevelopment of infants born to HIV-1-infected drug-using women	*Neurology*	Cohort spanned years <2000); Focused on opiates and cocaine
113	2011	Malee, K.M. *et al*	Mental health functioning among children and adolescents with perinatal HIV infection and perinatal HIV exposure	*AIDS Care*	Age >5 years (7-16yrs)
114	2016	Malee, K.M. *et al*	Brain and Cognitive Development Among U.S. Youth With Perinatally Acquired Human Immunodeficiency Virus Infection	*Journal of the Pediatric Infectious Diseases Society*	Review
115	2011	Manfredi, A.K. *et al*	Newborn hearing screening in infants born to HIV-seropositive mothers	*J Soc Bras Fonoaudiol*	No distinct HEU group - 'HIV-exposed' group analysed which may include HIV-infected children
116	2014	Manji, K.P. *et al*	Effect of multivitamin supplementation on the neurodevelopment of HIV-exposed Tanzanian infants: a randomized, double-blind, placebo-controlled clinical trial	*J. Tropical Pediatrics*	Focus on multivitamin supplementation
117	2012	Manno, D. *et al*	Rich micronutrient fortification of locally produced infant food does not improve mental and motor development of Zambian infants: a randomised controlled trial	*British Journal of Nutrition*	No distinct HEU group - separated by child HIV status and maternal HIV status but not the combined HEU v. HU groupings
118	2018	Marques, K.C. *et al*	Motor coordination of children and adolescents with human immunodeficiency virus	*Ciência & Saúde*	Language: Portuguese; Age > 5 years
119	2012	Martinez, P.C. *et al*	Intellectual quotient score comparison between HIV-infected and HIV exposed children at the Peruvian national institute of child health, Lima Peru	*Retrovirology (Conference)*	Age >5 years (3 - 7 years and no stratification); Conference presentation
120	2012	McDonald, C. *et al*	Morbidity and undernutrition are associated with impaired neurodevelopment among HIV-exposed infants in Tanzania	*FASEB Journal. Conference: Experimental Biology*	Conference abstract of 2013 McDonald *et al* paper; Insufficient information for HEU analysis
121	2013	McDonald, C. *et al*	Effect of multiple micronutrient supplementation on the neurodevelopment of HIV-exposed Tanzanian infants	*FASEB Journal. Conference: Experimental Biology*	Conference abstract of 2014 Manji *et al* paper.
122	2013	McDonald, C. *et al*	Stunting and wasting are associated with poorer psychomotor and mental development in HIV-exposed Tanzanian infants	*Journal of Nutrition*	Cohort spanned years <2000 (pregnant women recruited 1995 to 1997)
123	2006	McGrath, N *et al*	Effect of maternal multivitamin supplementation on the mental and psychomotor development of children who are born to HIV-1-infected mothers in Tanzania	*Pediatrics*	Cohort spanned years <2000 (pregnant women recruited 1995 to 1997); Not analysed by HEU status
124	2006	McGrath, N *et al*	The timing of mother-to-child transmission of human immunodeficiency virus infection and the neurodevelopment of children in Tanzania	*PIDJ*	Cohort spanned years <2000 (pregnant women recruited 1995 to 1997)
125	2018	McHenry, M.S. *et al*	Neurodevelopment in Young Children Born to HIV-Infected Mothers: A Meta-analysis	*Pediatrics*	Review
126	2019	McHenry, M.S. *et al*	In utero exposure to HIV and/or antiretroviral therapy: a systematic review of preclinical and clinical evidence of cognitive outcomes	*JIAS*	Review

127	2019	McHenry, M.S. *et al*	Interventions for developmental delays in children born to HIV-infected mothers: a systematic review	*AIDS Care*	Review
128	2018	McHenry, M.S. *et al*	Early childhood development in children born to HIV-infected mothers: perspectives from Kenyan clinical providers and caregivers	*Glob Pediatr Health*	No neurodevelopmental outcomes measured
129	2018	Mebrahtu, H. *et al*	Postpartum maternal mental health is associated with cognitive development of HIV-exposed infants in Zimbabwe: a cross-sectional study	*AIDS Care*	No ART exposure documented. Children with HIV included in the analysis
130	2019	Mebrahtu, H. *et al*	Effects of parenting classes and economic strengthening for caregivers on the cognition of HIV-exposed infants: a pragmatic cluster randomized controlled trial in rural Zimbabwe	*BMJ Global Health*	Developmental outcomes not assessed by HEU status; same trial [CHIDO] as included Mebrahtu *et al* 2018 paper
131	2019	Mebrahtu, H. *et al*	The impact of common mental disorders among caregivers living with HIV on child cognitive development in Zimbabwe	*AIDS Care*	Developmental outcomes not assessed by HEU status; same trial [CHIDO] as included Mebrahtu *et al* 2018 paper
132	2018	Mellins, C.A. *et al*	Screening for mental health among young South African children: the use of the Strengths and Difficulties Questionnaire (SDQ)	*Global Social Welfare*	Developmental outcomes not assessed by HEU status
133	2017	Milligan, R. *et al*	Working memory profiles in HIV-exposed, uninfected and HIV-infected children: A comparison with neurotypical controls	*Frontiers in Human Neuroscience*	Age >5years (mean age HEU group 88.28 months)
134	2019	Moraka, N.O. *et al*	Child HIV exposure and CMV seroprevalence in Botswana: No associations with 24-month growth and neurodevelopment	*Open Forum Infectious Diseases*	Analysis by CMV status and not HEU status; Children from the Tshipidi study included in Chaudhury *et al* paper
135	2013	Muhangi, L. *et al*	Maternal HIV infection and other factors associated with growth outcomes of HIV-uninfected infants in Entebbe, Uganda	*Public Health Nutrition*	No neurodevelopmental outcomes
136	2019	Mukherjee, S.B. *et al*	Development, cognition, adaptive function and maladaptive behavior in HIV-infected and HIV-exposed uninfected children aged 2-9 years	*Indian Pediatrics*	Age includes >5 years (2-9 years; HEU group mean 6.1 years) and no stratification
137	2017	Munoz, M *et al*	Community-Based Needs Assessment of Neurodevelopment, Caregiver, and Home Environment Factors in Young Children Affected by HIV in Lima, Peru	*Journal of the International Association of Providers of AIDS Care*	7 HEU children only and no HEU group analysis (only child and maternal status analysed separately)
138	2018	Murthy, V. *et al*	A study of neuropsychological profile of human immunodeficiency virus-positive children and adolescents on antiretroviral therapy	*Indian Journal of Psychiatry*	No specified HEU group; Age >5 years (8-15 years)
139	2017	Nachega, J.B. *et al*	Safety of tenofovir disoproxil fumarate-based antiretroviral therapy regimens in pregnancy for HIV-infected women and their infants: A systematic review and meta-analysis	*JAIDS*	Review
140	2014	Ngoma, M. *et al*	No evidence of neurodevelopmental delay in HEU infants exposed to cART in utero and breastfeeding	*Topics in Antiviral Medicine*	Conference presentation; duplicate of Ngoma *et al* 2014 paper included in analysis
141	2014	Ngoma, M. *et al*	Zambian HIV-Exposed Uninfected (HEU) infants exposed to HAART during pregnancy and one year of breastfeeding show no evidence of neurodevelopmental delay compared to HIV-Unexposed Uninfected (HUU) infants from the same community	*Canadian Journal of Infectious Diseases and Medical Microbiology*	Conference presentation; duplicate of Ngoma *et al* 2014 paper included in analysis
142	2012	Nielsen-Saines, K. *et al*	Infant outcomes after maternal antiretroviral exposure in resource-limited settings	*Pediatrics*	Included children with HIV in the analysis
143	2019	Obiagwu, P.N	Gross motor developmental delay in human immunodeficiency virus-infected children under 2 years of age	*Annals of African Medicine*	No HEU group (children were tested for HIV but mothers were only tested if children were HIV-infected)
144	2019	Onyango-Makumbi, C.	Extended prophylaxis with nevirapine does not affect growth in HIV-exposed infants	*JAIDS*	No maternal ART exposure comparison group (compared postnatal prophylaxis regimen)
145	2019	Pamplona, M.C.C.A. *et al*	Influence of exposure and vertical transmission of HIV-1 on the neuropsychomotor development in children'	*Revista da Sociedade Brasileira de Medicina Tropical*	No distinct HEU group (children born to mothers with HIV-1 infection compared to those born to mothers without HIV-1 infection, at least one HIV-infected child included in exposed group)
146	2010	Patel, D. *et al*	Breastfeeding, HIV status and weights in South African children: a comparison of HIV-exposed and unexposed children	*AIDS*	No neurodevelopmental outcomes
147	2005	Paul, M.E. *et al*	Morbidity and mortality during the first two years of life among uninfected children born to human immunodeficiency virus type 1-infected women: the women and infants transmission study	*PIDJ*	Cohort spanned years <2000 (enrolled by 1999)
148	2018	Paul, R. *et al*	Cognition, emotional health, and immunological markers in children with long-term nonprogressive HIV	*JAIDS*	Age >5 years (HEU median 6.8 [5.0-9.8]; HUU 7.4 [5.3 – 9.8])
149	2015	Perez, E.M. *et al*	Massage therapy improves the development of HIV-exposed infants living in a low socio-economic, peri-urban community of South Africa	*Infant Behavior & Development*	No HU control group or statistical comparison with normative data. Intervention study

150	2017	Pham, A. *et al*	Prenatal anti-retroviral exposure: An exploratory study of neurodevelopmental outcome in non-infected 5-years-old children	*European Journal of Paediatric Neurology*	Conference presentation, insufficient information
151	2018	Phillips, N.J. *et al*	HIV-associated cognitive disorders in perinatally infected children and adolescents: a novel composite cognitive domains score	*AIDS Care*	No specified HEU group; Age >5 year (9-12 years)
152	2016	Pierre, R.B. *et al*	Infectious disease morbidity and growth among young HIV-exposed uninfected children in Jamaica	*Pan American Journal of Public Health*	No neurodevelopmental outcomes
153	2018	Piske, M. *et al*	Developmental outcomes and ARV exposure in HIV-exposed uninfected children	*Topics in Antiviral Medicine*	Cohort spanned years <2000 (1990-2012); Age included >5 years. Conference abstract
154	2018	Piske, M. *et al*	Neurodevelopmental outcomes and in-utero antiretroviral exposure in HIV-exposed uninfected children	*AIDS*	Cohort spanned years <2000 (1990-2012); Model 3 presented children born between 1 April 2000 to 31 December 2012 but age included >5 years
155	2007	Potterton, J.L.	A longitudinal study of neurodevelopmental delay in HIV infected children	*NA*	Thesis; No distinct HEU group
156	2001	Potterton, J.L. & Eales, C.J.	Prevalence of developmental delay in infants who are HIV positive	*South African Journal of Physiotherapy*	No distinct HEU group
157	2016	Powis, K.M. *et al*	In-utero triple antiretroviral exposure associated with decreased growth among HIV-exposed uninfected infants in Botswana	*AIDS*	No neurodevelopmental outcomes
158	2011	Powis, K.M. *et al*	Effects of in utero antiretroviral exposure on longitudinal growth of HIV-exposed uninfected infants in Botswana	*JAIDS*	No neurodevelopmental outcomes
159	2018	Purswani, M. *et al*	Birth prevalence of congenital cytomegalovirus infection and language, hearing and developmental outcomes in a cohort of HIV-exposed, uninfected preschool children	*Open Forum Infectious Diseases*	Conference presentation of included paper *Purswani et al.*
160	2019	Purswani, M. *et al*	Birth prevalence of congenital cytomegalovirus infection in HIV-exposed uninfected children in the era of combination antiretroviral therapy	*JPEDS*	Focus on congenital cytomegalovirus
161	2010	Puthanakit, T. *et al*	Poor cognitive functioning of school-aged children in Thailand with perinatally acquired HIV infection taking antiretroviral therapy	*AIDS Patient Care and STDs*	Age > 5 years; (6-12 years)
162	2013	Puthanakit, T. *et al*	Cognitive function and neurodevelopmental outcomes in HIV-infected children older than 1 year of age randomized to early versus deferred antiretroviral therapy: The PREDICT neurodevelopmental study	*Paediatr Infect Dis J*	Age > 5 years and no stratification or sub-analysis (median 7 years). Of the 155 HEU children, 40 <5 years; of the 164 HU children, 38 <5 years.
163	2013	Ransom, C.E. *et al*	Infant growth outcomes after maternal tenofovir disoproxil fumarate use during pregnancy	*JAIDS*	No neurodevelopmental outcomes
164	2016	Redmond, S.M. *et al*	Longitudinal Evaluation of Language Impairment in Youth With Perinatally Acquired Human Immunodeficiency Virus (HIV) and Youth With Perinatal HIV Exposure	*Journal of the Pediatric Infectious Diseases Society*	Age >5 years (7 - 16 years)
165	2017	Reliquet, V. *et al*	Developmental delay and behavioral disorders in 59 HIV-exposed uninfected infants	*Translational Pediatrics*	Age includes >5 years and no sub-analysis (1.8-11.7 years follow up)
166	2016	Rice, M.L. *et al*	ARV risk for speech and language impairments in HEU children at 3 and 5 years	*Topics in Antiviral Medicine*	Conference presentation of included paper *Rice, M.L. et al 2018*
167	2005	Rocha, C. *et al*	Neurological findings in a group of children and adolescents exposed and infected by HIV-1.	*Arquivos de Neuro-Psiquiatria*	Language: Portuguese
168	2018	Rodriguez, V.J. *et al*	Pre- and postnatal exposure to intimate partner violence among South African HIV-infected mothers and infant developmental functioning at 12 months of age	*Archives of Women's Mental Health*	No distinct HEU group - 'HIV-exposed' group analysed which included 4 HIV-infected children'
169	2018	Rodriguez, V.J. *et al*	Infant development and pre- and post-partum depression in rural South African HIV-infected women	*AIDS & Behavior*	No distinct HEU group - 'HIV-exposed' group analysed which included 4 HIV-infected children'
170	2017	Rosala-Hallas, A. *et al*	Growth of HIV-exposed uninfected, compared with HIV-unexposed, Zambian children: a longitudinal analysis from infancy to school age	*BMC Pediatrics*	No neurodevelopmental outcomes
171	2019	Rotheram-Borus, M. J. *et al*	Maternal HIV does not affect resiliency among uninfected/HIV exposed South African children from birth to 5 years	*AIDS*	No disaggregation of results by HEU group – neurodevelopmental results presented for resilient group versus non-resilient group
172	2018	Rotheram-Fuller, E.J.	Maternal patterns of antenatal and postnatal depressed mood and the impact on child health at 3 years postpartum	*Journal of Consulting and Clinical Psychology*	No distinct HEU group - HIV-infected children excluded but analysis combined exposed and unexposed uninfected children
173	2019	Ruiseñor-Escudero, H. *et al*	Building capacity in neurodevelopment assessment of children in sub-Saharan Africa: A quality assurance model to implement standardized neurodevelopment testing	*Child Neuropsychol*	No results by HEU exposure presented; Age >5 years (mean across 6 sites 7.2 years)
174	2019	Ruiseñor-Escudero, H. *et al*	Neurodevelopmental outcomes in preschool children living with HIV-1 subtypes A and D in Uganda	*HIV reports*	No HU control group or statistical comparison with normative data
175	2016	Ruskowski, A. *et al*	The role of maternal vitamin D and iron status on developmental outcomes and head circumference in HIV-exposed uninfected infants	*Journal of Pediatric Gastroenterology and Nutrition*	Conference presentation; insufficient information

176	2015	Sa, C.S.C. *et al*	Motor and cognitive developmental of children exposed and no exposed to HIV	*Physiotherapy (United Kingdom)*	Conference abstract; included paper *da Silva et al, 2015*
177	2012	Salihu, H.M. *et al*	Maternal HIV/AIDS status and neurological outcomes in neonates: A population-based study	*Maternal and Child Health Journal*	Cohort spanned years <2000 (1998-2007)
178	2005	Sanmaneechai, O. *et al*	Growth, developmental, and behavioral outcomes of HIV-affected preschool children in Thailand	*Journal of the Medical Association of Thailand*	Population spanned years <2000 (children born 1998-2000)
179	2017	Shariat, M. *et al*	Growth and neurodevelopmental status in HIV infected children	*Iranian Journal of Pediatrics*	No HEU group
180	2007	Shaw, R.R.	The relationship between pediatric HIV infection, CD4 percentage, HAART, and WISC-III performance	*Dissertation Abstracts International Section A:*	Dissertation abstract; Insufficient information
181	2010	Shead, G.M. *et al*	Neurodevelopment and growth of institutionalized children with vertically transmitted human immunodeficiency virus	*Vulnerable Children and Youth Studies*	No HEU group (HIV-uninfected children, but no indication of maternal HIV status)
182	2014	Sherr, L. *et al*	A systematic review of psychological functioning of children exposed to HIV: using evidence to plan for tomorrow's HIV needs	*AIDS and behavior*	Review
183	2018	Sherr, L. *et al*	Cognitive and physical development in HIV-positive children in South Africa and Malawi: A community-based follow-up comparison study	*Child: Care, Health and Development*	Age includes >5 years (4-13 years) and no sub-analysis
184	2012	Shet, A. *et al*	Cognitive, neurological and adaptive behaviour functioning among children with perinatally-acquired HIV infection in India	*JIAS*	No distinct HEU group; Age includes >5 years (4-15 years); Conference abstract;
185	2014	Sibiude, J. *et al*	Association between prenatal exposure to antiretroviral therapy and birth defects: an analysis of the French perinatal cohort study (ANRS CO1/CO11)	*PLoS Medicine*	No HEU group analysis; Cohort spanned years <2000 (1994-2010).
186	2018	Siegle, C.B.H. & dos Santos Cardoso de Sa, C.	Concurrent validity between instruments of assessment of motor development in infants exposed to HIV	*Infant Behavior and Development*	No distinct HEU group – infants exposed to HIV which may include HIV-infected children
187	2014	Skeen, S. *et al*	Child development in HIV-positive and HIV-affected children in South Africa and Malawi-What role for community organisations?	*Children and Youth Services Review*	No specific HEU group analysis; Age > 5years (4-13 years)
188	2010	Smith, L. *et al*	Neurological and neurocognitive function of HIV-infected children commenced on antiretroviral therapy	*South African Journal of Child Health*	No HEU group
189	2014	Smith, M.L. *et al*	Neurocognitive development in young HIV-Exposed uninfected children exposed pre-or perinatally to antiretroviral medications	*Canadian Journal of Infectious Diseases and Medical Microbiology*	Conference abstract; appears to be overlap with 2017 paper included in full text reviews
190	2015	Smith, M.L. *et al*	Neurocognitive outcomes in pre-school and early school-age HIV-exposed uninfected children exposed pre-or perinatally to antiretroviral medications	*Canadian Journal of Infectious Diseases and Medical Microbiology*	Conference abstract; appears to be overlap with 2017 paper included in full text reviews
191	2005	Smith, R.	Mental functioning of children with HIV infection: The preschool and early school-age years	*Dissertation Abstracts International*	Age >5 years included (3-7 years); Dissertation
192	2006	Smith, R. *et al*	Effects of perinatal HIV infection and associated risk factors on cognitive development among young children	*Pediatrics*	Cohort spanned years <2000 (1990-2000 births); Age >5 years (3-7 years)
193	2014	Stein, A. *et al*	Predicting long-term outcomes for children affected by HIV and AIDS: Perspectives from the scientific study of children's development	*AIDS*	Review
194	2016	Strehlau, R. *et al*	HIV-associated neurodevelopmental delay: prevalence, predictors and persistence in relation to antiretroviral therapy initiation and viral suppression	Child: care, health and development	No HEU group
195	2018	Strehlau, R. *et al*	Neurodevelopmental assessment of HIV-exposed uninfected and early-treated HIV-infected children: study protocol	*BMC research notes*	Protocol; no results
196	2019	Strehlau, R. *et al*	Interventions addressing neurodevelopmental delay in young children infected with or exposed to HIV: A scoping review	*Rehabilitation Oncology*	Review
197	2020	Strehlau, R. *et al*	A description of early neurodevelopment in a cohort of HIV-exposed uninfected children	*AIDS Care*	No HU control group or statistical comparison with normative data.
198	2019	Sudfeld, C.R. *et al*	Third trimester vitamin D status is associated with birth outcomes and linear growth of HIV-exposed uninfected infants in the United Statues	*JAIDS*	Focus on vitamin D exposure
199	2006	Tahan, T.T. *et al*	Neurological profile and neurodevelopment of 88 children infected with HIV and 84 seroreverter children followed from 1995 to 2002	*Brazilian Journal of Infectious Diseases*	Cohort spanned years <2000 (1995-2002) with no stratification
200	2005	Tardieu, M. *et al*	Cerebral MR imaging in uninfected children born to HIV-seropositive mothers and perinatally exposed to zidovudine	*American Journal of Neuroradiology*	Cohort spanned years <2000
201	2017	Tassiopouolos, K. *et al*	Blood lead levels and neurodevelopmental function in perinatally HIV-exposed, uninfected children in a U.S.-based longitudinal cohort study	*AIDS Research and Human Retroviruses*	Focus on lead exposure

202	2010	Thomaidis, L. *et al*	Cognitive and psychosocial development of HIV pediatric patients receiving highly active anti-retroviral therapy: A case-control study	*BMC Pediatrics*	No HEU group; Age >5years (range 3-18 years)
203	2001	Thompson, W.S. *et al*	Language, memory, and cognitive performance in minority children infected with HIV (immune deficiency)	*Dissertation Abstracts International*	Dissertation; Age >5 years (school age); No distinct HEU group (uninfected siblings and peers)
204	2018	Tomlinson, M. *et al*	Antenatal depressed mood and child cognitive and physical growth at 18-months in South Africa: a cluster randomized controlled trial of home visiting by community health workers	*Epidemiology and psychiatric sciences*	No distinct HEU group
205	2015	Torre, P. III *et al*	Hearing assessment data in HIV-infected and uninfected children of Cape Town, South Africa	*AIDS Care*	Age includes >5 years (4-14 years) and no sub-analysis
206	2015	Torre, P. III *et al*	Distortion product otoacoustic emission data in perinatally HIV-infected and HIV-exposed but uninfected children and adolescents in the Pediatric HIV/AIDS Cohort Study	*Pediatr Infect Dis J*	Age > 5 years (7-16 years)
207	2012	Torre, P. III *et al*	Hearing loss in perinatally HIV-infected and HIV-exposed but uninfected children and adolescents	*Pediatr Infect Dis J*	Age >5 years (7-16yrs)
208	2008	Urban, M.F. *et al*	Growth of infants born to HIV-infected women when fed a biologically acidified starter formula with and without probiotics	*S Afr J Clin Nutr*	Trial confounded outcome
209	2016	Van Dalen, Y.W. *et al*	Neurometabolite Alterations Associated With Cognitive Performance in Perinatally HIV-Infected Children	*Medicine*	No specified HEU group; Age >5 years (8-18 years)
210	2019	Van den Hof, M. *et al*	Lower IQ and poorer cognitive profiles in treated perinatally HIV-infected children is irrespective of having a background of international adoption	*PLOS ONE*	No specified HEU group; Age >5 years (mean 10.45 years)
211	2020	Van den Hof, M. *et al*	Neurocognitive development in perinatally human immunodeficiency virus-infected adolescents on long-term treatment, compared to healthy matched controls: a longitudinal study	*CID*	No specified HEU group; Age >5 years (8-18 years)
212	2016	Van Dyke, R.B. *et al*	The PHACS SMARTT study: Assessment of the safety of in utero exposure to antiretroviral drugs	*Frontiers in Immunology*	Review of SMARTT outcomes
213	2019	Vannappagari, V. *et al*	Pregnancy and neonatal outcomes following prenatal exposure to dolutegravir	*JAIDS*	No neurodevelopmental outcomes, reports birth defects
214	2017	Van Wyhe, K.S. *et al*	Cross-cultural assessment of HIV-associated cognitive impairment using the Kaufman assessment battery for children: a systematic review	*JIAS*	Review
215	2018	Visser, M.J. *et al*	A comparative study of the psychological problems of HIV-infected and HIV-uninfected children in a South African sample	*AIDS Care*	No HEU group; age >5 years (6-12 years)
216	2003	Von Giesen, H.J. *et al*	Delayed motor learning and psychomotor slowing in HIV-infected children	*Neuropediatrics*	No specified HEU group; Age >5 years (8-16 years)
217	2018	Wesevich, A. *et al*	PMTCT option b+ efavirenz and tenofovir exposure through breastfeeding and bayleys neurodevelopmental scores in Malawian infants	*Pediatrics*	No specified HEU group - infants born to HIV-positive mothers which may include HIV-infected children; Conference presentation
218	2019	White, M. & Connor, K.L.	Determining how in utero HIV exposure, with or without infection, influences neurodevelopment in infants before age three: Findings from an evidenced-based review of observational and experimental studies	*Reproductive Sciences Supplement*	Review; Conference presentation
219	2019	White, M. *et al*	Does the early nutritional environment and in utero HIV exposure, without infection, impact infant development?	*Reproductive Sciences Supplement*	Conference presentation; no HEU and neurodevelopment analysis results
220	2012	Whitehead, N.	The neurodevelopment of HIV positive infants on HAART compared to HIV exposed but uninfected infants	*NA*	Thesis.
221	2014	Whitehead, N *et al*	The neurodevelopment of HIV-infected infants on HAART compared to HIV-exposed but uninfected infants	*AIDS Care*	No HU control group or statistical comparison with normative data.
222	2010	Williams, P.L. *et al*	Neurodevelopment and in utero antiretroviral exposure of HIV-exposed uninfected infants	*Pediatrics*	Cohort spanned years <2000 (1993-2006)
223	2012	Williams, P.L. *et al*	A trigger-based design for evaluating the safety of in utero antiretroviral exposure in uninfected children of human immunodeficiency virus-infected mothers	*American Journal of Epidemiology*	Methods paper, no neurodevelopmental results
224	2016	Williams, P.L. *et al*	Antiretroviral exposure during pregnancy and adverse outcomes in HIV-exposed uninfected infants and children using a trigger-based design	*AIDS*	Age >5 years included.
225	2019	Williams, P.L. *et al*	Associations of maternal ARV use with microcephaly in HIV-exposed uninfected children	*Open Forum Infectious Diseases*	Conference presentation, paper included in full text
226	2017	Yadav, S.K. *et al*	Altered structural brain changes and neurocognitive performance in pediatric HIV	*NeuroImage*	No HEU group; Age >5 years (controls mean 11.2 years)
227	2019	Yang, L. *et al*	Child development in HIV exposed, uninfected children: Challenges with accessing services	*Paediatrics and Child Health*	Conference abstract; insufficient information on developmental outcomes

S6 Appendix: List of included studies and relevant articles

	Study name *(where available)*	Country	HEU v. HU articles	ART analysis articles	Secondary outcomes articles
1.	-	Canada	Alimenti *et al*., 2006		
2.	***Malaria RCT***	Uganda	Boivin *et al*., 2016		
3.	**PROMISE**	Malawi & Uganda	Boivin *et al*., 2019	Boivin *et al*., 2019	Aizire *et al*., 2020
4.	**Rakai Community Cohort Study** (RCCS); **Nevirapine (NVP) Prevention of Mother to Child HIV Transmission (PMTCT) study**	Uganda	Brahmbhatt *et al.*, 2014		
5	**Tshipidi**	Botswana	Chaudhury *et al*., 2017	Chaudhury *et al*., 2018 (also Mma Bana)	
6	-	Brazil	Da Silva *et al*., 2017		
7	**PreNAPS PostNAPS**	Uganda	Familiar *et al*., 2018	Familiar *et al*., 2018	
8	-	Colombia	Gomez *et al*., 2009		Gomez *et al*., 2009
9	**HIVNET 012 clinical trial**	Zimbabwe	Kandawasvika *et al*., 2011		
10	-	Malawi	Landes *et al*., 2012		
11	**CHER**	South Africa	Laughton *et al*., 2012 Laughton *et al*., 2018		Laughton *et al*., 2012
12	**MCH-ART**	South Africa	Le Roux *et al*., 2018		Le Roux *et al*., 2019 (also HU2)
13	**Aluvia**	Zambia	Ngoma *et al*., 2014		
14	**SHINE**	Zimbabwe	Ntozini *et al*., 2020		
15	**SMARTT**	USA & Puerto Rico	Sirois *et al*., 2013	Caniglia *et al*., 2016 Rice *et al*., 2013 Rice *et al*., 2018 Sirois *et al*., 2013	Caniglia *et al*., 2016 Siberry *et al*., 2012 Jacobson *et al*., 2017 Williams *et al*., 2019
16	-	South Africa	Springer *et al*., 2012		Springer *et al*., 2012
17	**MIHS**	South Africa	Springer *et al*., 2018 Springer *et al*., 2020	Springer *et al*., 2018	Springer *et al*., 2018 Springer *et al*., 2020
18	-	Malawi	Struyf *et al*., 2019		
19	**-**	DRC	Van Rie *et al*, 2008 Van Rie *et al*, 2009		
20	**DCHS**	South Africa	Wedderburn *et al*., 2019		Donald *et al*., 2017 Tran *et al*., 2016
21	-	China	Wu *et al*., 2019		
22	**PMTCT RCT**	South Africa		Alcaide *et al*., 2019	
23	**Mpepu**	Botswana		Cassidy *et al*., 2019 (also Tshipidi and Mma Bana)	
24	**Mma Bana**	Botswana		Kacanek *et al*., 2018 (also Cassidy *et al*., 2019	
25	-	India		Rajan *et al*., 2017	
26	-	Canada		Smith *et al*., 2017	
27	-	Nigeria			Jumare *et al*., 2019
28	**CIGNIS**	Zambia			Filteau *et al*., 2011
29	-	USA			Neri *et al*., 2013
30	**Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) International Site Development Initiative (NISDI) study**	Latin America and Caribbean			Spaulding *et al*., 2016
31	-	Kenya			Pintye *et al*., 2015

S7 Appendix: Characteristics of studies examining neurodevelopment of HEU children compared to HU children

Author, year of publication, study	Country (time period)	Study design	Child age	N participants HEU	N participants HU	HIV test in HEU children (age)	Maternal ART (%)	ART regimen	Infant PNP (duration)	Breastfeeding	Tool	Confounders included in adjusted analysis
Alimenti *et al*., 2006[3]	Canada (2003 – 2004)	CS	18 – 36m	39	24	PCR (twice <6m) & Ab confirming seroreversion (6m)	100%	Triple therapy (quadruple in 3) AZT at delivery	AZT (neonatal period)	ND	BSID-II VABS	Maternal substance use.
Boivin *et al*., 2016[4]	Uganda (2010 – 2013)	Cohort / RCT	2yr	143	325	PCR at enrolment and (6w after cessation BF)	100%	Triple therapy	NVP (6wks)	100%; Duration HEU<HU	MSEL	Malaria, anaemia, age, WAZ, breastfeeding days, trial arm, sex, SES, observation days prior to randomization. Note RCT malaria intervention trial.
			3yr	122	331							
Boivin *et al*., 2019[5]	Malawi and Uganda (2013 – 2014)	Cohort/ RCT	12, 24, 48, and 60m	405	456	PCR (12m, 24m)	54% 46%	Triple therapy AZT	NVP (50%) None (50%) but maternal triple ART	HEU 73-83%, HU 95% 12m; Duration HEU<HU)	MSEL KABC-II	HEU and HU children matched for age, sex, SES. Data collection site.
Brahmbhatt *et al*, 2014[6]	Uganda (not reported)	Cohort	0 to 6yr (median 36.1m HEU 57.5m HU)	105	108	PCR (<18m), EIA (>18 m)	ND	NVP	NVP (% ND)	ND	MSEL	Child age, weight and height.
Chaudhury *et al*., 2017[7]	Botswana (2010 – 2012)	Cohort	2yr (22-29 m)	313 (BSID)	357 (BSID)	PCR (<18m) ELISA (18m)	36%	Triple therapy	NVP (single dose) and AZT (1m)	HEU 9% HU 99.5% HEU<HU	BSID-III - adapted	Maternal age, income, education, and depression, household access to water, sanitation and electricity, food insecurity, housing type, cooking method. Prematurity & birthweight examined in sensitivity analyses.
				337 (DMC)	386 (DMC)		64%	AZT			DMC	
Da Silva *et al*., 2017[8]	Brazil (timeframe ND)	CS	4, 8, 12 & 18m	40 (10/age)	40 (10/age)	PCR (16m)	100%	Not specified	Not specified (6wks)	Interrupted	BSID-III	–
Familiar *et al*., 2018[9]	Uganda (2012 – 2015)	LG	6 & 12m	75 complete data (79 total)	140 complete data (149 total)	DBS PCR	76% 24%	Triple therapy None	ND	100%	MSEL	Child sex, maternal age, education level, marital status, asset index, employment status, social support and depression, HOME score. HAZ mediator.
Gomez *et al*., 2009[10]	Colombia (not reported)	LG	0 – 24 m (3, 6, 9, 12 , 18 and 24m)	23	20	ELISA (18m)	100%	Triple therapy	ND	ND	BSID-II DDST	–
Kandawasvika *et al*., 2011[11]	Zimbabwe (2002 – 2004)	Cohort	3 to 12m (3m assessments)	188	287	PCR (<15m), Ab (>15mo)	In labour % ND	NVP	ND	99%	BINS	–
Landes *et al*., 2012[12]	Malawi (2008)	Cohort	20m	128	200	Health passport, offered rapid testing	~10% ~70%	Triple therapy NVP	NVP (66%)	HEU & HU>90% Duration HEU<HU	Milestones	–
Laughton *et al*., 2012[13] **CHER**	South Africa (2005 – 2006)	Cohort	11m (10-16m) 11.5m HEU 11.5m HU)	28	34	HEU: PCR at baseline and 1 month after PCV 3rd dose (12-24 wks).	ND	ART/PMTCT prophylaxis - personal correspondence	NVP and AZT (7 days) (%ND)	ND	GMDS	–
Laughton *et al*., 2018[14] **CHER**	South Africa (2005 – 2013)	Cohort	11 to 60m (11, 20, 30, 42, 60 m)	34	39	HEU: PCR at baseline and 1 month after PCV 3rd dose (12-24 wks).	85% 15%	PMTCT (mainly NVP and AZT) Unknown	NVP and AZT (85%)	ND	GMDS VMI	–
Le Roux *et al*., 2018 [15]	South Africa (2013 – 2016)	Cohort	13m (IQR 12-14)	215	306	PCR (12m)	100%	Triple therapy (TDF+FTC+ EFV)	NVP +/- AZT	100% (duration HEU<HU)	BSID-III (no receptive language) OFC	Gestation, sex, SGA, Maternal education, intimate partner violence, Risky drinking, breastfeeding duration, housing, maternal age, employment; planned pregnancy; postpartum depression
Ngoma *et al*., 2014[16]	Zambia (2011 – 2013)	Double cohort	15 to 36m Mean HEU 22.4m HU 24.1	97	103	RNA PCR on DBS; Controls had serology performed.	100% In labour	Triple therapy (ZDV+3TC+ LPV/r) NVP in labour	NVP (48h) and AZT (1wks)	100% (duration HEU<HU)	FSDQ (CAT/CLAMS)	Child age and birthweight, maternal education, monthly income.

Ntozini *et al*., 2020[17]	Zimbabwe (2016 – 2017)	RCT	24m	205	1175	PCR / Rapid (18m)	86% 24%	ART (majority TDF-based triple therapy) Unknown	ND	100%	MDAT CDI A-not-B	(1): Child age, sex and month of birth, trial arm, study nurse. (2): maternal education, household wealth, maternal age, parity.
Sirois *et al*., 2013[18]	USA & Puerto Rico (2007 – 2011)	Cohort	12.7m (9 to 15m)	374	49	ND in paper; study design on clinicaltrials.gov	97% 2% 1%	Triple therapy Other None	AZT (8w) 97%	ND	BSID-III	–
Springer *et al*., 2012[19]	South Africa (2009) (Pilot study)	CS	17 to 19m	17	20	PCR (2, 6 & 12wks)	94%	ART/ PMTCT prophylaxis	ND	HEU 6%, HU 100%	GMDS OFC	–
Springer *et al*., 2018[20] **MIHS**	South Africa (2012 – 2013)	Nested cohort	12m (11-14)	58	38	ELISA with PCR if positive (12m)	50% 50%	cART AZT monotherapy	NVP and AZT (1wks)	HEU 12%, HU 66% at 6m	BSID-III ADBB OFC	–
Springer *et al*., 2020[21] **MIHS**	South Africa (2012 – 2013)	Cohort	3yrs (30-42 m)	32	27	ELISA with PCR if positive (12m)	41% 59%	cART AZT	NVP and AZT (1wks)	HEU 9%, HU 67% at 6m	BSID-III SDQ OFC	Stunting, maternal education.
Struyf *et al*., 2019[22]	Malawi (2008 – 2011)	Cohort	15wks to 24m (BSID at 15w, 6, 9, 12, 15, 18 and 24m)	289	170	PCR (6w and follow up visits), Ab (>18mo) also for HU. Note 21/289 seroconverted and were censored from analysis	29.5%	NVP only	NVP (90%)	ND	BSID-III Cognitive only	–
Van Rie et al, 2008[23] **(DRC cohort)**	Democratic Republic of Congo (DRC) (2004-2005)	CS	18m to 72m (HEU median 33.4 m; HU median 45.6m)	35	90	HIV-uninfected children who were orphans of maternal AIDS / whose mothers had symptomatic AIDS	ND	Unclear but ART was available at the time	ND	ND	BSID (18-29m) PDMS SONR (30-72m) RITLS (18-36m)	–
Van Rie *et al*., 2009[24] **(DRC cohort)**	DRC (2004 – 2005)	Cohort	18m to 71m	35	90	*As above.* ELISA available.	ND	Unclear but ART was available at the time	ND	ND	BSID-II PDMS SONR	–
Wedderburn *et al*., 2019[25]	South Africa (2012 – 2015)	Cohort	6m	61	199	PCR at 6 weeks, PCR/Ab (9m/18m)	88%	Triple therapy	NVP 87%; NVP+AZT 13%	HEU 14%, HU 18% at 6m. Duration HEU<HU	BSID-III	Child age and sex, maternal age and education, household income. Sensitivity analysis prematurity, depression, breastfeeding.
			2yr	168	564		12%	AZT				
Wu *et al*., 2019[26]	China (2010 – 2013)	CS	6m to 3yr in five age bands	250 (50/age)	250 (50/age)	PCR (6wks)	100%	Triple therapy	AZT or NVP (4 to 6w) (100%)	ND but guidelines HEU FF (~97.9%)	BSID-III	HEU and HU children matched for child gender, age, maternal age and residency. Neonatal jaundice, child anaemia, low birthweight, prematurity and malnutrition, maternal education and smoking.

Abbreviations | ND: not documented; HEU: children who are HIV-exposed and uninfected; HU: children who are HIV-unexposed; CS: cross-sectional study; LG: longitudinal study; RCT: randomized controlled trial; DRC: Democratic Republic of Congo; yr: year; m: months; wks: weeks; ART: antiretroviral treatment; PNP: postnatal prophylaxis; AZT: zidovudine; 3TC: lamivudine; NVP: nevirapine; LPV/r: lopinavir/ritonavir; TDF: tenofovir: FTC: emtricitabine; EFV: efavirenz; cART: combination ART; PMTCT: prevention of mother-to-child transmission; Ab: HIV antibody test; PCR: HIV polymerase chain reaction test; DBS: Dried blood spot; FF: formula feeding; OFC: occipitofrontal circumference; HCAZ: head circumference-for-age; NP: neonatal period; WAZ: weight for age; SES: socioeconomic status; SGA: small for gestational age; STI: sexually transmitted infection; All assessment tool abbreviations are listed in S8 Appendix.

S8 Appendix: Child development tools used across included studies highlighting those in the meta-analysis

Tool	Abbreviation	Scales	Domains included in meta-analysis
A-not-B task	A-not-B	Object permanence; early executive	
Alarm Distress Baby Scale	ADBB	Socioemotional state	
Ages & Stages Questionnaire	ASQ	Cognitive, language, motor,	
Bayley Infant Neurodevelopmental Screener	BINS	Risk for developmental delay	
Bayley Scales of Infant & Toddler Development, 2nd edition	BSID-II	Mental & psychomotor development	
Bayley Scales of Infant & Toddler Development, 3rd edition	BSID-III	Cognitive, receptive language, expressive language, fine motor, gross motor, social-emotional,	Cognitive, receptive language, expressive language, fine motor, gross motor
Beery-Buktenica developmental tests of Visual Motor Integration	VMI	Visual perception, motor, and hand-eye coordination	
Capute Scales Clinical Adaptive Test: Cognitive Adaptive Test	CAT	Cognition, visual-motor	
Capute Clinical Linguistic and Auditory Milestone Scales	CLAMS	Language and nonverbal problem-solving skills	
Denver Developmental Screening Test	DDST	Gross motor, language, fine motor, adaptive, and personal-social,	
Development Assessment Scale for Indian Infants	DASII	Motor and mental scales	
Developmental Milestones Checklist	DMC	Locomotor, fine motor, language, personal-social	
Dubowitz Neonatal Neurobehavioral Tool	DNNT	Neurobehaviour and neurological	
Full-Scale Developmental Quotient	FSDQ	Cognition, Language, Social	
Goldman-Fristoe Test of Articulation	GFTA	Language	
Griffiths Mental Development Scales	GMDS	Locomotor, personal-social, hearing & language, eye & hand coordination, performance	
Head circumference-for-age z score or Occipital frontal circumference	HCAZ or OFC		
Kaufman Assessment Battery for Children, 2nd edition	KABC-II	Simultaneous, sequential, learning, planning, knowledge	
MacArthur-Bates Communicative Developmental Inventories	CDI	Language by parent report	CDI vocabulary used for expressive language per correspondence with expert
Malawi Developmental Assessment Tool	MDAT	Total score, gross motor, fine motor, language, social	Total score, gross motor, fine motor, language - MDAT language used for receptive language per correspondence with expert; total score used to represent cognitive development
Mullen Scales of Early Learning	MSEL	Visual reception, fine motor, gross motor, receptive and expressive language, composite	Fine motor, gross motor, receptive and expressive language, composite - Early learning composite used to represent cognitive development
Peabody Developmental Motor Scales	PDMS	Motor	
Peabody Picture Vocabulary Test	PPVT	Language	
Personal, social and emotional development	PSED	Social-emotional development	
Rossetti Infant-Toddler Language Scale	RITLS	Language	
Strengths and Difficulties Questionnaire	SDQ	Socio-emotional	
Snijders-Oomen Nonverbal Intelligence Test	SONR	Intelligence	
Test of early language development	TELD	Receptive and expressive language	
Vineland Adaptive Behaviour Scales	VABS	Daily living, socialization, communication, motor	
WHO Milestones Chart	Milestones	Motor, language	
Wechsler Preschool and Primary Scales of Intelligence	WPPSI	Verbal IQ, Performance IQ, Full Scale IQ and General Language	

S9 Appendix: Quality assessment and risk of bias of studies comparing HEU and HU children

Study authors	Year	1. Objective stated	2. Study population defined and representative*	3. Participation rate is >50%*	4. Control subjects are from the same community*	5a. Sample size calculation	5b. Sample is >50 subjects per group	6. Exposure is measured before outcome#	7. Timeframe is sufficient to see the exposure effects#	8. Additional analyses performed (ART/VL/CD4)	9. A valid HIV test is used in mothers	10. A valid HIV test is used in children	11. Valid neurodevelopment tests are used in children**	12. Assessors are blinded to child HIV exposure status**	13. Lost to follow up is <20%^	14. Potential confounders adjusted for (or matched)#	Quality assessment
Alimenti *et al.*	2006	✓	✓	?	✗	✓	✗	✗	✗	✓	✓	✓	✓	✓	NA	✓	FAIR
Boivin *et al.*	2016	✓	?	✓	✓	✗	✓	✓	✓	✗	✓	✓	✓	?	✗	✓	FAIR
Boivin *et al.*	2019	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	?	✓	✓	GOOD
Brahmbhatt *et al.*	2014	✓	✗	?	?	✗	✓	✓	✓	✗	✓	✓	✓	?	NA	✓	FAIR
Chaudhury *et al.*	2017	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	?	✓	✓	GOOD
da Silva *et al.*	2017	✓	✗	?	✓	✓	✗	✗	✗	✗	?	✓	✓	?	NA	✗	POOR
Familiar *et al.*	2018	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	?	?	✓	GOOD
Gomez *et al.*	2009	✓	✗	✗	?	✗	✗	✓	✓	✗	✓	✓	✓	?	✗	✗	POOR
Kandawasvika *et al.*	2011	✓	?	✓	?	✓	✓	✓	✓	✗	✓	✓	?	✓	?	✗	FAIR
Landes *et al.*	2012	✓	✓	✗	✓	✗	✓	✓	✓	✗	?	✓	?	?	✓	✗	POOR
Laughton *et al.*	2012	✓	✗	✗	✓	✗	✗	✓	✓	✗	✓	✓	✓	✓	NA	✗	FAIR
Laughton *et al.*	2018	✓	✗	✓	✓	✓	✗	✓	✓	✗	✓	✓	✓	✓	✗	✗	FAIR
le Roux *et al.*	2018	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	?	✗	✓	GOOD
Ngoma *et al.*	2014	✓	?	✓	✗	✗	✓	✗	✗	✗	✓	✓	✓	?	NA	✓	FAIR
Ntozini *et al.*	2020	✓	✓	✗	✓	✗	✓	✓	✓	✗	✓	✓	✓	?	✓	✓	GOOD
Sirois *et al.*	2013	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	?	✗	✗	GOOD
Springer *et al.*	2012	✓	✓	?	✓	✗	✗	✗	✗	✗	✓	✓	✓	✓	✗	✗	FAIR
Springer *et al.*	2018	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗	✗	GOOD
Springer *et al.*	2020	✓	✓	✗	✓	✗	✗	✓	✓	?	✓	✓	✓	✓	✗	?	FAIR
Struyf *et al.*	2019	✓	✓	?	✓	✓	✓	✓	✓	✓	✓	?	✓	?	✗	✗	FAIR
van Rie *et al.*	2008	✓	✗	?	✗	✗	✗	✗	✗	✗	✗	?	✓	?	NA	✗	POOR
van Rie *et al.*	2009	✓	✗	?	✗	✗	✗	✓	✓	✗	✗	?	✓	?	?	✗	POOR
Wedderburn *et al.*	2019	✓	✓	?	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	?	✓	GOOD
Wu *et al.*	2019	✓	✓	✗	?	✗	✓	✗	✗	✗	✓	✓	✓	?	NA	✓	FAIR

✓Yes; ✗No; ?Not reported / Not documented / Cannot determine; NA: not applicable; ART: antiretroviral therapy; VL: viral load; CD4: CD4 cell count.
Types of bias: *Selection bias; **Information bias; ^Attrition bias; #Confounding

S10 Appendix: Characteristics of studies of HEU children comparing different maternal ART regimens

Author, year of publication	Country (time period)	Study design	Child age	Tool	Last HIV test (age)	Maternal ART regimen (Group A; *N*)	Maternal ART regimen (Group B; *N*)	Maternal ART regimen/control (Group C; *N*)	Infant PNP (duration)	Breast-feeding	Confounders included in adjusted analysis
Alcaide *et al*., 2019[27]	South Africa (2015 – 2018)	CS	9 to 20 m 13.4±1.9 HEU	BSID-III	PCR from DBS (12m)	Triple therapy, EFV/TDF/FTC detectable at 32 weeks' gestation (66)	Triple therapy, EFV/TDF/FTC undetectable (14)	-	ND	ND	Intimate partner violence, maternal depression.
Boivin *et al*., 2019*[5]	Malawi and Uganda (2013 – 2014)	Cohort / RCT	12m, 2, 4 & 5yr	MSEL KABC-II	PCR (12, 24 m)	Group 1 & 2 Antenatal triple ART (PI-based) + postnatal triple ART or infant NVP (93 / 103)	Groups 3 & 4 Antenatal AZT monotherapy + postnatal triple ART or infant NVP (88 / 80)	HU (374)	*See groups*	Yes	Data collection site. *Note:* PI-based regimen: LPV/r + 3TC + AZT or LPV/r + FTC + TDF
Caniglia *et al*., 2016[28] **SMARTT**	USA & Puerto Rico (2006 – 2013)	Cohort	9 to 15m	BSID-III OFC	ND	ART: ATV-based 1st trimester 2nd/3rd trimester	ART: no ATV 1st trimester 2nd/3rd trimester	-	ND	ND	Maternal education, CD4, HIV RNA, year, illicit substances, alcohol, tobacco, race, ethnicity, primary language, household income, age, Full Scale IQ, STI, LBW, GA.
						(Total N= 575)					
Cassidy *et al*., 2019[29] **Tshipidi plus (Mpepu; Mma Bana; Tshipidi)**	Botswana (2016 – 2017)	Cohort	2yr	BSID-III DMC PSED	Per individual cohort	Triple therapy, EFV/TDF/FTC (126)	Triple therapy, non-EFV-multiple (367)	-	NVP (single dose) and AZT (1m) or NVP(1m)	EFV 29%; Not EFV 73%	Child age, sex, feeding method, *in utero* ART initial exposure timing, maternal age, employment, income, marital status, indoor faucet, electricity, indoor toilet. Sensitivity analyses: Preterm birth, LBW.
Chaudhury *et al*., 2018[30] **Mma Bana** **Tshipidi**	Botswana (2006 – 2008) (2010 – 2012)	Cohort & RCT	2yr	BSID-III DMC	PCR at birth, 1, 6, 12 months. ELISA (>18mo)	ART: multiple regiments (382)	AZT monotherapy (210)	-	NVP (single dose) and AZT (1m)	ART 71.5% AZT 8.1%	Maternal age, education, income, CD4 cell count, year of neurodevelopmental testing. Sensitivity analysis restricted to formula fed infants from one cohort. Prematurity & birthweight examined in sensitivity analyses.
Familiar *et al*., 2018*[9]	Uganda (2012 – 2015)	Cohort	6 & 12m	MSEL	DBS PCR	ART: multiple regimens (57)	No therapy (18)	HU (140)	Not specified	Yes	Child age and sex, maternal age, education level, marital status, HOME, SES, social support and depression.
Kacanek *et al*., 2018[31] **Mma Bana**	Botswana (2006 – 2008)	CS/RCT	2yr	BSID-III DMC PSED	Per study: birth, 1m, 6m PCR	Triple NRTI ABC/AZT/3TC (101)	Dual NRTI+PI LVP/r / AZT/3TC (96)	-	NVP (single dose) and AZT (1m)	Yes	Low maternal body mass index at follow-up, child age, access to electricity in the home, GA at enrolment. Randomised from 26-34w gestation.
Rajan et al, 2017[32]	India (2013 – 2015)	Cohort	6-18 m (assessed at enrolment, 3m, 6m)	DASII	PCR (6 wks, 6 m, and 12 wks after breast feeding); Serology at 18 m	ART (31)	No ART (10)	-	NVP (single dose n=80), NVP (6 wks, n=30), none (n=3)	ND	-

Rice *et al*., 2013[33] **SMARTT**	USA & Puerto Rico (2007 – 2011)	Cohort	1 yr 2 yr	CDI ASQ	ND	cART PI regimen NNRTI (Total N=535 at 12m) (Total N=503 at 24m) Individual drugs (464 at 12m) (431 at 24m)	Non-cART No PI No NNRTI	-	AZT-alone; combination	ND	Child sex, age and language exposure, neonatal prophylaxis, maternal IQ, CD4 cell count, viral load during pregnancy, caregiver health problems.
Rice *et al*., 2018[34] **SMARTT**	USA & Puerto Rico (2007 – 2011)	Cohort	3 & 5yr	Speech: GFTA Language: TELD Language: PPVT	ND	Individual drugs (208 at 3y) (429 at 5y) Combination ART	Triple NRTI	-	ND	ND	Child age, sex, race and ethnicity, maternal education, caregiver health problems and alcohol use during pregnancy.
Sirois *et al*., 2013*[18] **SMARTT**	USA & Puerto Rico (2007 – 2011)	Cohort	12m (9 to 15m)	BSID-III	ND	Combination ART (>=3 drugs >= 2 classes) PI-containing (with or without NNRTI) NNRTI-containing (without PI) Individual drugs N=374	Non-combination ART NRTI only NRTI only		AZT (8wks) 97%	ND	Child age, year of delivery, sex, last viral load prior to delivery, STI during pregnancy, maternal full scale IQ score, substance use, maternal age at delivery. Sensitivity analyses: prematurity and SGA
Smith *et al*., 2017[35]	Canada ND	LG	3.5yr (5.5 yr not included in age range)	WPPSI VABS VMI	2+ DNA PCR assays or after 1 month negative HIV serology at any age	Triple therapy, PI-based (43)	Triple therapy, NNRTI-based (16)	-	AZT +/- NVP, 3TC	ND	-
Springer *et al*., 2018*[20]	South Africa (2012 – 2013)	Cohort	12m (11 to 14m)	BSID-III ADBB OFC	ELISA with PCR if positive (12m)	ART: multiple regimens (29)	AZT monotherapy (29)	HU (38)	NVP and AZT (1wks)	HEU 12% 6m, ND by ART	–

Abbreviations | ND: not documented; HEU: children who are HIV-exposed and uninfected; HU: children who are HIV-unexposed; CS: cross-sectional study; LG: longitudinal study; RCT: randomized controlled trial; yr: year; m: months; wks: weeks; PCR: HIV polymerase chain reaction test; DBS: Dried blood spot; ART: antiretroviral treatment; PNP: postnatal prophylaxis; AZT: zidovudine; 3TC: lamivudine; NVP: nevirapine; LPV/r: lopinavir/ritonavir; TDF: tenofovir: FTC: emtricitabine; EFV: efavirenz; ATV: atazanavir; ABC: abacavir; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; cART: combination ART; PMTCT: prevention of mother-to-child transmission; FF: formula feeding; NP: neonatal period; WAZ: weight for age; LBW: low birth weight; SES: socioeconomic status; GA: Gestational age; STI: sexually transmitted infection; All assessment tool abbreviations are listed in S8 Appendix.

*Also in HEU v. HU analysis

S11 Appendix: Characteristics of studies examining head circumference and neuroimaging

Author, year of publication	Country (time period)	Study design	Child age	N HEU	N HU	HIV test in HEU children (age)	Maternal ART (%)	Maternal ART regimen	Infant PNP (duration)	Breast-feeding	Confounders included in adjusted analysis
Aim 1: HEU children versus HU children - Head circumference											
Donald *et al*., 2017[36] **DCHS**	South Africa (2012 – 2015)	Cohort	Neonatal period	131	536	PCR at 6 weeks, PCR/Ab (9m/18m)	100%	Triple ART or AZT monotherapy	NPV or NVP+AZT	ND	Socioeconomic status, depression, smoking, alcohol use in interaction variables (HIV by alcohol use and HIV by smoking status).
Le Roux *et al*., 2019[37]	South Africa (2013 – 2016)	Cohort	Birth to 1yr (0, 3m, 6m, 9m, 12m)	461	411	PCR at 6 weeks and 48 weeks.	100%	Triple therapy	ND	Yes	Age-adjusted, sex-adjusted and gestation-adjusted z-scores. Socioeconomic factors, risky drinking, intimate partner violence, recent childhood illness, and infant feeding) with and without adjustment for birth outcomes.
Jumare *et al*., 2019[38]	Nigeria (2013 – 2017)	Cohort	Birth to 18m	297	103	PCR at birth, 3-4wks, 6wks, 1yr or 6wks postbreastfeeding	100%	Triple therapy	NVP (6w)	Yes	WHO standard z-scores. Maternal education, marital status, breastfeeding, prematurity, maternal weight and baseline Z-score.
Gomez *et al*., 2009*[10]	Colombia (not reported)	LG	Birth to 2yrs (0, 3, 6m, 9m, 12m, 18m, 2y)	23	20	ELISA (18m)	100%	Triple therapy, multiple	ND	ND	-
Filteau *et al*., 2011[39]	Zambia (2005 – 2009)	RCT	6m to 2 yrs	125	382	Ab testing at 18m	ND	NVP	NVP	HEU 42%, HU 97%	- (Only baseline characteristics included here)
Neri *et al*., 2013[40]	USA (2006 – 2009)	LG	2w to 2yrs (mean age 10m)	111 (82 in matched group)	82	PCR first 6 m, Ab after 12m	96%	Triple therapy	AZT (6w)	ND	Age, sex, race.
Springer *et al*., 2018*[20] **MIHS**	South Africa (2012 – 2013)	Cohort	12m (11-14)	58	38	ELISA with PCR if positive (12m)	50% 50%	cART AZT	ND	HEU no (12%), HU yes (66% at 6 months)	-
Aizire *et al*., 2020[41]	Malawi and Uganda (2013 – 2014)	Cohort	1 to 2yrs (1yr, 2yr)	471	462	Documentation of uninfected status	54% 45% 1%	Triple therapy AZT No ART	NVP in some	HEU Yes, HU Yes. HU>HEU	Breastfeeding status, maternal age, electricity/gas use and tap-water use.
Laughton *et al*., 2012*[13]	South Africa (2005 – 2006)	Cohort	11m (10-16m) 11.5m HEU 11.5m HU)	28	34	HEU: PCR at baseline and 1 month after PCV 3rd dose (12-24wks).	ND	NVP and AZT	ND. Likely NVP and AZT	ND	-
Springer *et al*., 2012*[19]	South Africa (2009)	CS	17 to 19m	17	20	PCR (2, 6 & 12wks)	94%	PMTCT prophylaxis or cARV	ND	HEU no, HU yes	-
Springer *et al*., 2020*[21] **MIHS**	South Africa (2012 – 2013)	Cohort	2 to 3yr (30 to 42m)	32	27	ELISA with PCR if positive (12m)	40.6% 59.4%	cART AZT	NVP and AZT (1w)	HEU low (9%), HU (67%) at 6 months	-

Aim 1: HEU children versus HU children - Neuroimaging											
Tran *et al*., 2016[42] **DCHS**	South Africa (2012 – 2015)	Cohort	Neonatal period	15	22	PCR at 6 weeks, PCR/Ab (9m/18m)	100%	Triple ART	NVP / NVP & AZT	ND	Neonatal postnatal age and infant sex.
Aim 2: ART analyses - Head circumference				**Group A**	**Group B**						
Spaulding *et al*., 2016[43]	Latin America and Caribbean (2002 – 2009)	Cohort	Birth to 6m (0-3m, 6m)	1400 Multiple ART	-	HIV virologic assay at 1m and 4m or older or HIV Ab after 6 m	67% 32% 2%	PI + 2 NRTIs 2 NRTIs + NNRTI *Other/non-adherent*	AZT	ND; likely none	Maternal and infant demographics and delivery-, infant-, and maternal HIV-, and infant HIV treatment-related covariates including congenital and infant infections, obstetric complications, and maternal infections were considered.
Pintye *et al*., 2015[44]	Kenya (2013)	CS	6w and 9m	TDF+ 51	TDF- 104	PCR testing	100%	Triple therapy, multiple	ND	Yes	Maternal age, education level, time since HIV diagnosis, infant breastfeeding, gestational age, maternal WHO clinical stage, timing of ART initiation, trimester of first use of 3-drug combination ART and PI-containing ART regimen.
Siberry *et al*., 2012[45] **SMARTT**	USA (2005-2010)	Cohort	Birth and 1yr	TDF+ 274 209	TDF- 416 361	ND	100%	Triple therapy, multiple	ND	No	Infant sex, household income, maternal viral load, maternal tobacco use during pregnancy, birth cohort, race, gonorrhoea infection. Sensitivity analysis including GA.
Caniglia *et al*., 2016[28] ***SMARTT***	USA & Puerto Rico (2006 – 2013)	Cohort	1yr	ART: ATV+ N=127	ART: ATV- N=525	ND	100%	ART, multiple	ND	ND	Maternal education, CD4, HIV RNA, year, illicit substances, alcohol, tobacco, race, ethnicity, primary language, household income, age, Full Scale IQ, STI, LBW, GA, trimester of ART initiation.
Jacobson *et al*., 2017[46] **SMARTT**	USA & Puerto Rico (2007 – 2011)	Cohort	2yr	509 Multiple ART	–	ND	100%	Triple therapy, multiple	ND	ND	Region, alcohol, tobacco, income, language at home.
Williams *et al*., 2020[47] **SMARTT**	USA & Puerto Rico (2007 – 2017)	Cohort	Birth to 5yrs	3055 Multiple ART	-	ND	141 2842	EFV+ non-EFV+ Multiple combinations assessed	ND	ND	Education, household income, alcohol use during pregnancy, birth cohort.

Abbreviations | ART: antiretroviral treatment; AZT: zidovudine; EFV: efavirenz; TDF: tenofovir; NVP: nevirapine; 3TC: lamivudine; FTC: emtricitabine; cART: combination ART; PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; BF: breastfeeding; NP: neonatal period; PNP: postnatal prophylaxis; HEU: Children who are HIV-exposed and uninfected; HU: Children who are HIV-unexposed; CS: cross-sectional study; LG: longitudinal study; RCT: randomized controlled trial; HCAZ: head-circumference-for-age z-score; ND: not documented

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Appendix VII: Supplementary Information Chapter 7

Neuroimaging Young Children and Associations with Neurocognitive Development in a South African Birth Cohort Study *(research paper)*

Supplementary Material

Supplementary data for Wedderburn *et al*., Neuroimaging young children and associations with neurocognitive development in a South African birth cohort study.

Appendix Table 1: Comparison of socioeconomic variables among children in the neuroimaging sub-group and the full birth cohort (n=1143)

Variables	Final analysis group (n=146)			Full imaging cohort (n=239)		
	Imaging (n=146)	No Imaging (n=997)	*P*	Imaging (n=239)	No Imaging (n=904)	*P*
Male sex	84 (57.5%)	504 (50.6%)	0.115	135 (56.5%)	453 (50.1%)	0.079
Monthly household income (ZAR)						
< R1000 (<~$75)	48 (32.9%)	383 (38.4%)		78 (32.6%)	353 (39.1%)	
R1000-R5000 (~$75-375)	86 (58.9%)	471 (47.2%)		139 (58.2%)	418 (46.2%)	
>R5000 (>~$375)	12 (8.2%)	143 (14.3%)	0.017*	22 (9.2%)	133 (14.7%)	0.003*
Maternal education						
Primary	7 (4.8%)	79 (7.9%)		14 (5.9%)	72 (8.0%)	
Secondary	91 (62.3%)	518 (52.0%)		143 (59.8%)	466 (51.6%)	
Completed secondary	40 (27.4%)	335 (33.6%)		70 (29.3%)	305 (33.7%)	
Any tertiary	8 (5.5%)	65 (6.5%)	0.115	12 (5.0%)	61 (6.8%)	0.134
Maternal employment status (employed)	41 (28.1%)	266 (26.7%)	0.721	67 (28.0%)	240 (26.6%)	0.645
SES quartile						
Lowest SES	29 (19.9%)	245 (24.6%)		46 (19.3%)	228 (25.2%)	
Low-mod SES	35 (24.0%)	261 (26.2%)		66 (27.6%)	230 (25.4%)	
Mod-high SES	45 (30.8%)	245 (24.6%)		69 (28.9%)	221 (24.5%)	
High SES	37 (25.3%)	246 (24.7%)	0.334	58 (24.3%)	225 (24.9%)	0.200

Footnotes:
*p<0.05; Chi-squared for categorical variables (n and % proportions presented).

Appendix Table 2: Comparison of socioeconomic variables among children in the neuroimaging sub-group and those in DCHS follow up at 2 years (n=1002)

Variables	Imaging & Neurodevelopment (n=146)			Full imaging cohort (n=239)		
	Imaging (n=146)	No Imaging (n=856)	*P*	Imaging (n=239)	No Imaging (n=763)	*P*
Male sex	84 (57.5%)	429 (50.1%)	0.097	135 (56.5%)	378 (49.5%)	0.061
Monthly household income (ZAR)						
< R1000 (<~$75)	48 (32.9%)	338 (39.5%)		78 (32.6%)	308 (40.4%)	
R1000-R5000 (~$75-375)	86 (58.9%)	406 (47.4%)		139 (58.2%)	353 (46.3%)	
>R5000 (>~$375)	12 (8.2%)	112 (13.15)	0.028*	22 (9.2%)	102 (13.4%)	0.005*
Maternal education						
Primary	7 (4.8%)	66 (7.7%)		14 (5.9%)	59 (7.7%)	
Secondary	91 (62.3%)	458 (53.5%)		143 (59.8%)	406 (53.2%)	
Completed secondary	40 (27.4%)	281 (32.8%)		70 (29.3%)	251 (32.9%)	
Any tertiary	8 (5.5%)	51 (6.0%)	0.219	12 (5.0%)	47 (6.2%)	0.324
Maternal employment status (employed)	41 (28.1%)	223 (26.1%)	0.607	67 (28.0%)	197 (25.8%)	0.498
SES quartile						
Lowest SES	29 (19.9%)	213 (24.9%)		46 (19.3%)	196 (25.7%)	
Low-mod SES	35 (24.0%)	233 (27.2%)		66 (27.6%)	202 (26.5%)	
Mod-high SES	45 (30.8%)	208 (24.3%)		69 (28.9%)	184 (24.1%)	
High SES	37 (25.3%)	202 (23.6%)	0.256	58 (24.3%)	181 (23.7%)	0.182

Footnotes:
*p<0.05; Chi-squared for categorical variables (n and % proportions presented).

Appendix Table 3: Associations of anatomical ROIs (cortical surface area and thickness) with either cognitive or language development adjusting for household income

			Cognitive development (n=146)		Language development (n=138)	
Cortical Surface Area	**Lobe**	**Hemisphere**	**Beta coefficient (95% CI)**	***P***	**Beta coefficient (95% CI)**	***P***
Fusiform	Temporal	L	0.04 (-0.17 to 0.26)	0.685	0.27 (0.06 to 0.49)**	0.013*
		R	0.15 (-0.06 to 0.37)	0.157	0.26 (0.04 to 0.48)**	0.023*
Insula	Temporal	L	-0.03 (-0.25 to 0.20)	0.815	0.09 (-0.14 to 0.33)	0.440
		R	0.09 (-0.11 to 0.28)	0.398	0.21 (0.01 to 0.41)**	0.043*
Lateral orbitofrontal	Frontal	L	0.13 (-0.11 to 0.37)	0.282	0.25 (0.00 to 0.49)**	0.048*
		R	0.10 (-0.14 to 0.34)	0.421	0.28 (0.04 to 0.52)**	0.024*
Paracentral	Frontal	L	-0.03 (-0.22 to 0.17)	0.785	0.12 (-0.08 to 0.32)	0.226
		R	-0.18 (-0.37 to 0.00)	0.051	-0.12 (-0.31 to 0.07)	0.216
Cortical Thickness	**Lobe**	**Hemisphere**	**Beta coefficient (95% CI)**	***P***	**Beta coefficient (95% CI)**	***P***
Caudal middle frontal	Frontal	L	-0.22 (-0.38 to -0.07)**	0.005*	-0.17 (-0.33 to -0.01)	0.034*
		R	-0.14 (-0.30 to 0.02)	0.075	-0.12 (-0.28 to 0.05)	0.158
Lateral orbitofrontal	Frontal	L	-0.02 (-0.18 to 0.14)	0.829	-0.01 (-0.17 to 0.15)	0.891
		R	-0.12 (-0.28 to 0.04)	0.126	-0.19 (-0.35 to -0.03)	0.017*
Medial orbitofrontal	Frontal	L	-0.19 (-0.35 to -0.03)	0.021*	-0.20 (-0.36 to -0.04)**	0.012*
		R	-0.18 (-0.34 to -0.02)	0.032*	-0.29 (-0.45 to -0.13)**	0.001*
Rostral middle frontal	Frontal	L	-0.17 (-0.33 to -0.01)	0.038*	-0.10 (-0.27 to 0.06)	0.225
		R	-0.14 (-0.30 to 0.02)	0.083	-0.20 (-0.36 to -0.04)**	0.018*
Superior parietal	Parietal	L	-0.15 (-0.32 to 0.01)	0.063	-0.22 (-0.38 to -0.06)**	0.009*
		R	0.02 (-0.15 to 0.19)	0.856	-0.08 (-0.26 to 0.09)	0.333
Supramarginal	Parietal	L	-0.04 (-0.20 to 0.12)	0.618	-0.05 (-0.22 to 0.12)	0.562
		R	-0.20 (-0.36 to -0.04)**	0.014*	-0.16 (-0.32 to 0.00)	0.056

Footnote

Table showing the ROIs with associations for cognitive and language development with cortical surface area and cortical thickness, if either hemisphere had $p<0.05$. All linear regression models included child age, child sex and household income as covariates; associations with surface area also included intracranial volume. The beta (standardised) regression coefficient represents the effect size or expected change in cognitive or language development (in standard deviations) with a one unit standard deviation change in the region-of-interest. Beta coefficients are reported to 2 decimal places. *$p<0.05$, **absolute beta coefficient>=0.20

Appendix Table 4: Associations of anatomical ROIs (cortical surface area and thickness) with either cognitive or language development (excluding outliers)

			Cognitive development (n=144)		Language development (n=136)	
Cortical Surface Area	**Lobe**	**Hemisphere**	**Beta coefficient (95% CI)**	***P***	**Beta coefficient (95% CI)**	***P***
Fusiform	Temporal	L	0.02 (-0.20 to 0.24)	0.862	0.28 (0.06 to 0.50)**	0.014*
		R‡	0.15 (-0.08 to 0.38)	0.199	0.28 (0.05 to 0.52)**	0.019*
Insula	Temporal	L	-0.05 (-0.28 to 0.18)	0.676	0.08 (-0.16 to 0.32)	0.503
		R	0.07 (-0.13 to 0.28)	0.486	0.19 (-0.01 to 0.40)	0.067
Lateral orbitofrontal	Frontal	L	0.12 (-0.13 to 0.36)	0.350	0.22 (-0.03 to 0.46)**	0.089
		R	0.08 (-0.17 to 0.33)	0.522	0.27 (0.02 to 0.52)**	0.032*
Paracentral	Frontal	L	-0.06 (-0.26 to 0.15)	0.579	0.11 (-0.10 to 0.31)	0.302
		R	-0.20 (-0.39 to -0.01)**	0.035*	-0.12 (-0.31 to 0.07)	0.221
Cortical Thickness	**Lobe**	**Hemisphere**	**Beta coefficient (95% CI)**	***P***	**Beta coefficient (95% CI)**	***P***
Caudal middle frontal	Frontal	L	-0.23 (-0.39 to -0.07)**	0.006*	-0.18 (-0.34 to -0.01)	0.034*
		R	-0.12 (-0.29 to 0.04)	0.129	-0.11 (-0.27 to 0.06)	0.198
Lateral orbitofrontal	Frontal	L	0.00 (-0.16 to 0.16)	0.993	0.00 (-0.16 to 0.17)	0.968
		R	-0.11 (-0.27 to 0.05)	0.189	-0.19 (-0.35 to -0.03)	0.020*
Medial orbitofrontal	Frontal	L	-0.16 (-0.32 to -0.00)	0.049*	-0.20 (-0.36 to -0.04)**	0.015*
		R	-0.16 (-0.32 to 0.01)	0.064	-0.29 (-0.45 to -0.12)**	0.001*
Rostral middle frontal	Frontal	L	-0.14 (-0.30 to 0.02)	0.095	-0.09 (-0.26 to 0.07)	0.263
		R	-0.12 (-0.28 to 0.04)	0.154	-0.19 (-0.35 to -0.03)	0.021*
Superior parietal	Parietal	L	-0.11 (-0.27 to 0.06)	0.196	-0.19 (-0.35 to -0.02)	0.026*
		R	0.03 (-0.14 to 0.20)	0.705	-0.09 (-0.26 to 0.09)	0.329
Supramarginal	Parietal	L	-0.00 (-0.17 to 0.16)	0.960	-0.03 (-0.19 to 0.14)	0.736
		R	-0.18 (-0.34 to -0.02)	0.028*	-0.15 (-0.31 to 0.01)	0.073

Footnote

Table showing the ROIs with associations for cognitive and language development with cortical surface area and cortical thickness, if either hemisphere had $p<0.05$. All linear regression models included child age and child sex as covariates; associations with surface area also included intracranial volume. The beta (standardised) regression coefficient represents the effect size or expected change in cognitive or language development (in standard deviations) with a one unit standard deviation change in the region-of-interest. ‡Right fusiform has n=143 for the cortical surface area calculation as one outlier was excluded. *$p<0.05$, **absolute beta coefficient>=0.20

Appendix VIII: Supplementary Information Chapter 8

Early Structural Brain Development in Infants Exposed to HIV and Antiretroviral Drugs *In Utero* in a South African Birth Cohort *(research paper)*

Supplementary Information

Early structural brain development in infants exposed to HIV and antiretroviral therapy *in utero* in a South African birth cohort

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Supplementary Methods

Neuroimaging acquisition details

3D T2-weighted MR images were acquired at the Cape Universities Brain Imaging Centre (CUBIC), Tygerberg Hospital, Cape Town using a Siemens Magnetom 3T Allegra MRI scanner (Erlangen, Germany). Infants were fed, swaddled in a blanket, and encouraged to sleep. A qualified nurse or pediatrician remained in the scanner room with the infant at all times, and a pulse oximeter monitored pulse and oxygenation throughout the scan. Given the challenges scanning neonates, a radiofrequency transmit/receive head coil was used with a wet clay inlay (40x40cm, 2cm thickness, standard sculpting clay) and voltage was decreased to optimize signal. Sagittal T2-weighted images were acquired using the following parameters: repetition time (TR) = 3500ms; echo time (TE) = 354 ms; FOV = 160 x 160 mm; in-plane resolution= 1.3 x 1.3 x 1.0mm, 128 slices. Sequence scan time was 5 minutes 41 seconds.

Neuroimaging processing

Images were first converted from DICOM to NIfTI format using the dcm2nii conversion tool. NIfTI images were then brain extracted using the FSL 5.0 brain extraction tool (BET) [1]. Each scan was visually checked following the initial BET and additional thresholds were applied to further improve the brain extraction.

Acquired structural T2-weighted images were processed using Statistical Parametric Mapping Software (SPM8) (www.fil.ion.ucl.ac.uk/spm/software/spm8) run in Matlab R2017B, using the University of North Carolina (UNC) custom infant T2 template in Montreal Neurological Institute (MNI) standard space [2]. Data from 95 infants (39 females, 56 males) were used to create this template and the associated tissue probability maps served as priors for segmentation. The UNC infant atlas has demonstrated improved performance when compared to manual segmentation as well as other comparable atlases [2-4]. In brief, the T2 images were registered to the UNC infant template and subsequently spatially normalised, where bias corrections and smoothing were applied as per the

SPM8 default settings. Images were segmented into grey matter, white matter, and cerebrospinal fluid using tissue priors as per the UNC infant template, and the resulting probabilistic maps were saved for further analysis [2]. During preprocessing, T2 images were modulated to preserve the volumetric information despite relative volumetric increases and decreases during image warping.

Neuroimaging data quality assessment

The normalized images and segmented grey matter maps were visually inspected by two researchers for segmentation accuracy and proper alignment to the template. Where there was disagreement over alignment quality, a third researcher reviewed the scans and made a final decision. Any images that failed normalization (n=4), or segmentation (n=13) were discarded. Masking and extraction were performed using the Masking toolbox [5]. Outlier detection was performed using the ENIGMA protocol (http://enigma.ini.usc.edu/protocols/imaging-protocols/) [6], to determine any values that were greater than 1.5 times the interquartile range. Any regions marked as a statistical outlier were re-inspected to evaluate segmentation.

Sensitivity analyses

Sensitivity analyses were performed (1) excluding imaging outliers (per the ENIGMA guidelines); (2) restricting analyses to children ≤28 days old; (3) restricting the group to only those children from one recruitment clinic, as this was closely correlated to HIV status; (4) including maternal prenatal alcohol use, smoking and depression, as these differed between the groups, and may be potential confounders; and (5) excluding children who tested positive for CMV at any point.

Table S1: Sociodemographic characteristics of children with neuroimaging versus those without in the Drakenstein Child Health Study

Variables	DCHS	Neonatal imaging versus no imaging		
	All children (n=1141)†	Imaging (n=146)	No imaging (n=995)	*P*
HIV exposure	247 (21.7%)	40 (27.4%)	207 (20.8%)	0.071
Male sex	586 (51.4%)	74 (50.7%)	512 (51.5%)	0.862
Site (Mbekweni)	632 (55.4%)	73 (50.0%)	559 (56.2%)	0.161
Monthly household income (ZAR)				
< R1000 (<~$75)	431 (37.8%)	49 (33.6%)	382 (38.4%)	
R1000-R5000 (~$75-375)	556 (48.7%)	75 (51.4%)	481 (48.3%)	
>R5000 (>~$375)	154 (13.5%)	22 (15.1%)	132 (13.3%)	0.514
Maternal education				
Primary	85 (7.5%)	7 (4.8%)	78 (7.8%)	
Secondary	609 (53.4%)	73 (50.0%)	536 (53.9%)	
Completed secondary	374 (32.8%)	58 (39.7%)	316 (31.8%)	
Any tertiary	73 (6.4%)	8 (5.5%)	65 (6.5%)	0.201
Maternal employment status (employed)	307 (26.9%)	40 (27.4%)	267 (26.8%)	0.886
Relationship status (married / cohabitating)	460 (40.4%)	63 (43.5)	397 (39.9)	0.416
Maternal age at birth, years	26.0 (22.3 - 31.1)	26.9 (22.0 – 31.6)	26.0 (22.3 – 31.0)	0.304
Gestational age at delivery, weeks	39 (38 – 40)	39 (38 – 40)	39 (37 – 40)	0.054
Birthweight, g	3025 (604)	3157 (462)	3005 (620)	0.025*
Maternal smoking during pregnancy				
Active	351 (32.2%)	45 (31.0%)	306 (32.4%)	
Passive	479 (43.9%)	61 (42.1%)	418 (44.2%)	
Non-smoker	261 (23.9%)	39 (26.9%)	222 (23.5%)	0.667
Maternal alcohol use during pregnancy	131 (13.2%)	25 (17.9%)	106 (12.4%)	0.077
Maternal depression	238 (23.9%)	44 (31.4%)	194 (22.6%)	0.023*
Maternal and child HIV variables (HEU children)				
Maternal HIV diagnosis timepoint				
Before pregnancy	174 (72.2%)	27 (71.1%)	147 (72.4%)	
During pregnancy	67 (27.8%)	11 (29.0%)	56 (27.6%)	0.864
Maternal CD4 cell count				
Median (range) (cells/mm^3)	411 (285 – 594)	423 (286 – 594)	408 (283 – 593)	0.925
<350 cells/mm^3	80 (40.2%)	13 (39.4%)	67 (40.4%)	
350-500 cells/mm^3	44 (22.1%)	8 (24.2%)	36 (21.7%)	
>500 cells/ mm^3	75 (37.7%)	12 (36.4%)	63 (38.0%)	0.948
Maternal Viral load (VL) in pregnancy				
Lower than detectable limit (<40 copies/mL)	93 (64.1%)	19 (73.1%)	74 (62.2%)	
VL detectable (40-1000 copies/mL)	30 (20.7%)	5 (19.2%)	25 (21.0%)	
Virally unsuppressed (>1000 copies/mL)	22 (15.2%)	2 (7.7%)	20 (16.8%)	0.450

Antiretroviral drug initiation				
Before conception	96 (40.0%)	15 (37.5%)	81 (40.5%)	
During pregnancy	144 (60.0%)	25 (62.5%)	119 (59.5%)	0.724
Antiretroviral regimen during pregnancy				
Monotherapy with AZT (zidovudine)	36 (15.0%)	5 (12.5%)	31 (15.6%)	
2 NRTIs + NNRTI (1st line non-EFV)	25 (10.4%)	3 (7.5%)	22 (11.0%)	
2 NRTIs + NNRTI (1st line EFV-containing)	166 (69.2%)	31 (77.5%)	135 (67.5%)	
2 NRTIs + PI (2nd / 3rd line)	13 (5.4%)	1 (2.5%)	12 (6.0%)	0.609
Infant prophylaxis				
NVP (nevirapine) alone	210 (87.1%)	35 (87.5%)	175 (87.1%)	
NVP + AZT	31 (12.9%)	5 (12.5%)	26 (12.9%)	0.940

Legend: Data are n/N (%), mean (SD) or median (IQR). Continuous variables were compared with unpaired t-tests or Mann-Whitney U tests; categorical variables were compared with Chi-squared. *$p<0.05$. †Full DCHS cohort excluding two children with HIV-infection. All HIV-related variables are cited out of the number of HIV-infected mothers with available data. Percentages are cited among those with non-missing values. Missing data: relationship status (n=1); maternal age (n=3); smoking (n=50); alcohol (n=146); depression (n=143); birthweight (n=11); gestational age (n=5); Maternal HIV diagnosis timepoint (n=6); CD4 cell count (n=48); Viral load (n=102); ART timepoint (n=7); ART regimen (n=7); Infant prophylaxis (n=6); Specific variables were assessed as follows: (i) Maternal smoking levels by urine cotinine, ≥500 ng/ml quantified active smoking, 10-500 mg/ml as passive smoking and <10ng/ml as non-smoking. (ii) Maternal alcohol use assessed and quantified using the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) and retrospectively collected data on moderate-severe alcohol use in pregnancy forming a dichotomous measure; (iii) Maternal depression in pregnancy measured using the Edinburgh postnatal depression scale (EPDS), a threshold of ≥13 was used for depression; (iv) The lowest maternal CD4 during pregnancy was used to reflect maternal immunosuppression in pregnancy; (v) Maternal viral load was categorised into <40 copies/ml as lower than the detectable limit, ≥40-<1000copies/ml as detectable and ≥1000copies/ml as unsuppressed. Where there was more than one result, the highest viral load during pregnancy was taken. Abbreviations: HEU = HIV-exposed and uninfected; HU = HIV-unexposed; VL = viral load; NVP = nevirapine; AZT = zidovudine, EFV = efavirenz; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor

Table S2: Adjusted mean differences in grey matter volumes according to HIV exposure status excluding statistical outliers

Brain volumes	*N*		Mean (SD) volume (mm^3)		Minimally adjusted† coefficient (95% CI)	P-value	Effect size	Fully Adjusted‡ coefficient (95% CI)	P-value	Effect size
	HEU	HU	HEU	HU			Cohen's d (95% CI)			Cohen's d (95% CI)
Global volume										
Total grey matter	39	104	234,393 (14,983)	239,041 (11,898)	-4365 (-8510 to -220)	0.039*	-0.33 (-0.70 to 0.04)	-4305 (-8567 to -43.95)	0.048*	-0.33 (-0.70 to 0.04)
Subcortical regions										
Thalamus	38	102	3214 (106)	3214 (90)	5.22 (-30.06 to 40.49)	0.770	0.06 (-0.32 to 0.43)	5.94 (-30.12 to 42.00)	0.745	0.06 (-0.31 to 0.44)
Caudate	39	103	1786 (157)	1893 (153)	-105.54 (-161.48 to -49.59)	0.0003*§	-0.63 (-1.01 to -0.25)	-104.50 (-161.52 to -47.48)	0.0004*§	-0.62 (-1.00 to -0.25)
Putamen	37	101	2833 (70)	2843 (75)	-8.43 (-36.27 to 19.40)	0.550	-0.11 (-0.49 to 0.26)	-7.71 (-36.46 to 21.04)	0.597	-0.11 (-0.48 to 0.27)
Pallidum	38	101	714 (27)	713 (28)	1.64 (-8.51 to 11.78)	0.750	0.06 (-0.31 to 0.43)	1.09 (-9.35 to 11.52)	0.837	0.04 (-0.33 to 0.41)
Hippocampus	38	104	1582 (141)	1606 (123)	-13.76 (-57.37 to 29.85)	0.534	-0.11 (-0.48 to 0.26)	-13.43 (-58.19 to 31.33)	0.554	-0.10 (-0.48 to 0.27)
Amygdala	36	104	543 (23)	542 (21)	1.87 (-5.59 to 9.32)	0.621	0.09 (-0.29 to 0.47)	1.99 (-5.69 to 9.68)	0.608	0.09 (-0.29 to 0.47)

Legend: Subcortical volumes (mean of left and right hemispheres), mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes for associations between brain volumes and HIV-exposure excluding outliers. Outliers were dropped bilaterally per region if either hemisphere was an outlier. Total intracranial volume outliers were dropped for all analyses. Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. †Minimally adjusted models included child age at scan, child sex and intracranial volume. ‡Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income, and maternal age. Linear regression models where negative estimates indicate HIV exposure is associated with lower volumes in that region. *Uncorrected $p<0{\cdot}05$. § p-values survive multiple comparison correction using the false discovery rate across subcortical regions. Abbreviations: HEU = HIV-exposed and uninfected; HU = HIV-unexposed; CI = Confidence Interval.

Table S3: Adjusted mean differences in grey matter volumes according to HIV exposure status restricted to children ≤28 days

Brain volumes	Mean (SD) volume (mm^3)		Minimally adjusted† coefficient (95% CI)	P-value	Effect size	Fully Adjusted‡ coefficient (95% CI)	P-value	Effect size
	HEU (n=34)	HU (n=87)			Cohen's d (95% CI)			Cohen's d (95% CI)
Global volume								
Total grey matter	233969 (14815)	238812 (12086)	-4796 (-9322 to -270)	0.038*	-0.36 (-0.76 to 0.04)	-4998 (-9671 to -325)	0.036*	-0.38 (-0.78 to 0.02)
Subcortical regions								
Thalamus	3208 (112)	3220 (94)	-8.08 (-47.61 to 31.45)	0.686	-0.08 (-0.48 to 0.32)	-9.13 (-49.54 to 31.29)	0.655	-0.09 (-0.49 to 0.30)
Caudate	1799 (158)	1884 (155)	-83.89 (-146.70 to -21.09)	0.009*	-0.51 (-0.91 to -0.11)	-93.35 (-157.86 to -28.84)	0.005*§	-0.57 (-0.97 to -0.16)
Putamen	2837 (88)	2839 (84)	-3.48 (-36.87 to 29.92)	0.837	-0.04 (-0.44 to 0.36)	-0.75 (-35.53 to 34.04)	0.966	-0.01 (-0.41 to 0.39)
Pallidum	710 (34)	714 (29)	-4.74 (-16.51 to 7.03)	0.427	-0.16 (-0.55 to 0.24)	-4.38 (-16.76 to 8.01)	0.485	-0.14 (-0.54 to 0.25)
Hippocampus	1577 (145)	1611 (120)	-24.29 (-71.86 to 23.29)	0.314	-0.19 (-0.59 to 0.21)	-36.87 (-84.75 to 11.00)	0.130	-0.29 (-0.68 to 0.11)
Amygdala	537 (33)	543 (21)	-4.45 (-13.64 to 4.73)	0.339	-0.18 (-0.58 to 0.22)	-5.43 (-14.93 to 4.07)	0.260	-0.22 (-0.62 to 0.18)

Legend: Subcortical volumes (mean of left and right hemispheres), mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes for associations between brain volumes and HIV-exposure restricted to children ≤28 days (n=121). Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. †Minimally adjusted models included child age at scan, child sex and intracranial volume. ‡Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income, and maternal age. Linear regression models where negative estimates indicate HIV exposure is associated with lower volumes in that region. *Uncorrected p<0·05. ¶p-values survive multiple comparison correction using the false discovery rate across subcortical regions. Abbreviations: HEU = HIV-exposed and uninfected; HU = HIV-unexposed; CI = Confidence Interval.

Table S4: Adjusted mean differences in grey matter volumes according to HIV exposure status restricted to Mbekweni clinic

Brain volumes	Mean (SD) volume (mm^3)		Minimally adjusted† coefficient (95% CI)	P-value	Effect size	Fully Adjusted‡ coefficient (95% CI)	P-value	Effect size
	HEU (n=39)	HU (n=34)			Cohen's d (95% CI)			Cohen's d (95% CI)
Global volume								
Total grey matter	233,675 (15,602)	238,679 (12,069)	-5730 (-10836 to -624)	0.028*	-0.40 (-0.86 to 0.07)	-5684 (-10941 to -427)	0.035*	-0.39 (-0.86 to 0.07)
Subcortical regions								
Thalamus	3204 (114)	3187 (98)	19.86 (-29.54 to 69.26)	0.425	0.19 (-0.27 to 0.65)	18.67 (-30.18 to 67.52)	0.448	0.18 (-0.28 to 0.64)
Caudate	1781 (154)	1840 (144)	-65.70 (-133.76 to 2.37)	0.058	-0.42 (-0.89 to 0.04)	-73.30 (-139.98 to -6.62)	0.032*	-0.47 (-0.94 to -0.00)
Putamen	2832 (86)	2839 (93)	-11.08 (-50.58 to 28.41)	0.577	-0.12 (-0.58 to 0.34)	-13.99 (-53.05 to 25.08)	0.477	-0.16 (-0.62 to 0.30)
Pallidum	711 (33)	713 (29)	-4.43 (-18.61 to 9.76)	0.536	-0.14 (-0.60 to 0.32)	-5.13 (-19.25 to 9.00)	0.471	-0.16 (-0.63 to 0.30)
Hippocampus	1564 (164)	1557 (132)	7.40 (-45.16 to 59.95)	0.780	0.05 (-0.41 to 0.51)	6.35 (-46.18 to 58.89)	0.810	0.04 (-0.42 to 0.50)
Amygdala	535 (32)	540 (23)	-5.24 (-16.78 to 6.30)	0.368	-0.18 (-0.65 to 0.28)	-5.32 (-17.24 to 6.60)	0.376	-0.19 (-0.65 to 0.27)

Legend: Subcortical volumes (mean of left and right hemispheres), mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes for associations between brain volumes and HIV-exposure restricted to children attending Mbekweni clinic (n=73). Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. †Minimally adjusted models included child age at scan, child sex and intracranial volume. ‡Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income, and maternal age. Linear regression models where negative estimates indicate HIV exposure is associated with lower volumes in that region. *Uncorrected p<0·05. Abbreviations: HEU = HIV-exposed and uninfected; HU = HIV-unexposed; CI = Confidence Interval.

Table S5: Grey matter volumes according to HIV exposure status assessing the effect of maternal depression on the exposure-outcome relationship

Brain volumes	Minimally adjusted† coefficient (95% CI)	P-value	Effect size Cohen's d (95% CI)	Fully Adjusted‡ coefficient (95% CI)	P-value	Effect size Cohen's d (95% CI)
Global volume						
Total grey matter	-5388 (-9687 to -1089)	0.014*	-0.40 (-0.78 to -0.02)	-5032 (-9432 to -632)	0.025*	-0.37 (-0.75 to 0.01)
Subcortical regions						
Thalamus	14.33 (-26.15 to 54.82)	0.485	0.13 (-0.25 to 0.51)	17.07 (-24.58 to 58.71)	0.419	0.16 (-0.22 to 0.54)
Caudate	-91.05 (-150.53 to -31.56)	0.003*§	-0.54 (-0.92 to -0.16)	-87.39 (-147.29 to -27.49)	0.005*§	-0.52 (-0.90 to -0.14)
Putamen	-0.17 (-32.33 to 31.99)	0.992	-0.002 (-0.38 to 0.38)	0.12 (-33.11 to 33.35)	0.994	0.001 (-0.38 to 0.38)
Pallidum	0.10 (-11.55 to 11.75)	0.986	0.003 (-0.38 to 0.38)	0.77 (-11.26 to 12.81)	0.899	0.02 (-0.35 to 0.40)
Hippocampus	-16.56 (-62.92 to 29.81)	0.481	-0.12 (-0.50 to 0.26)	-12.96 (-60.63 to 34.71)	0.592	-0.09 (-0.47 to 0.29)
Amygdala	-1.46 (-9.90 to 6.98)	0.733	-0.06 (-0.44 to 0.32)	-0.24 (-8.92 to 8.44)	0.956	-0.01 (-0.39 to 0.37)

Legend: Mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes for associations between brain volumes and HIV-exposure accounting for antenatal maternal depression. Antenatal maternal depression was measured using the Edinburgh Postnatal Depression Scale (EPDS) where a cut-off score of ≥13 was used as the threshold for depression. Depression was used as a proxy for maternal psychosocial illness; however, further work needs to be done to assess this potential mediator. Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. †Minimally adjusted models included child age at scan, child sex, intracranial volume, and maternal depression during pregnancy. ‡Fully adjusted models included child age and child sex, maternal education, household income, maternal age, and maternal depression. Linear regression models where negative estimates indicate HIV exposure is associated with lower volumes in that region. *Uncorrected p<0·05. § p-values survive multiple comparison correction using the false discovery rate across subcortical regions. Abbreviation: CI = Confidence Interval.

Table S6: Grey matter volumes according to HIV exposure status assessing the effect of smoking on the exposure-outcome relationship

Brain volumes	Minimally adjusted† coefficient (95% CI)	P-value	Effect size Cohen's d (95% CI)	Fully Adjusted‡ coefficient (95% CI)	P-value	Effect size Cohen's d (95% CI)
Global volume						
Total grey matter	-5188 (-9338 to -1037)	0.015*	-0.39 (-0.75 to -0.02)	-5264 (-9509 to -1019)	0.015*	-0.39 (-0.76 to -0.02)
Subcortical regions						
Thalamus	6.09 (-32.98 to 45.17)	0.758	0.06 (-0.31 to 0.42)	8.75 (-31.46 to 48.97)	0.668	0.08 (-0.28 to 0.45)
Caudate	-90.79 (-149.47 to -32.10)	0.003*§	-0.54 (-0.91 to -0.17)	-83.51 (-142.40 to -24.61)	0.006*§	-0.50 (-0.87 to -0.13)
Putamen	-10.30 (-41.20 to 20.60)	0.511	-0.12 (-0.49 to 0.24)	-8.58 (-40.37 to 23.21)	0.594	-0.10 (-0.47 to 0.26)
Pallidum	-3.49 (-14.49 to 7.51)	0.532	-0.11 (-0.48 to 0.25)	-3.57 (-14.94 to 7.79)	0.535	-0.12 (-0.48 to 0.25)
Hippocampus	-26.54 (-72.21 to 19.12)	0.252	-0.19 (-0.55 to 0.18)	-27.52 (-74.63 to 19.58)	0.250	-0.20 (-0.56 to 0.17)
Amygdala	-3.46 (-11.83 to 4.90)	0.414	-0.14 (-0.51 to 0.22)	-3.33 (-12.02 to 5.36)	0.450	-0.14 (-0.50 to 0.23)

Legend: Mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes for associations between brain volumes and HIV-exposure accounting for maternal smoking during pregnancy. Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. †Minimally adjusted models included child age at scan, child sex, intracranial volume, and maternal smoking during pregnancy. ‡Fully adjusted models included child age and child sex, maternal education, household income, maternal age, and maternal smoking. Linear regression models where negative estimates indicate HIV exposure is associated with lower volumes in that region. *Uncorrected p<0·05. § p-values survive multiple comparison correction using the false discovery rate across subcortical regions. Abbreviation: CI = Confidence Interval.

Table S7: Grey matter volumes according to HIV exposure status assessing the effect of alcohol on the exposure-outcome relationship

Brain volumes	Minimally adjusted† coefficient (95% CI)	P-value	Effect size Cohen's d (95% CI)	Fully Adjusted‡ coefficient (95% CI)	P-value	Effect size Cohen's d (95% CI)
Global volume						
Total grey matter	-4493 (-8772 to -214)	0.040*	-0.33 (-0.71 to 0.05)	-4272 (-8637 to 94)	0.055	-0.32 (-0.70 to 0.06)
Subcortical regions						
Thalamus	8.17 (-32.60 to 48.93)	0.693	0.08 (-0.30 to 0.45)	12.16 (-29.58 to 53.90)	0.565	0.11 (-0.27 to 0.49)
Caudate	-103.84 (-164.40 to -43.27)	0.0009*§	-0.61 (-1.00 to -0.22)	-102.23 (-163.69 to -40.77)	0.001*§	-0.60 (-0.99 to -0.22)
Putamen	-1.01 (-33.27 to 31.26)	0.951	-0.01 (-0.39 to 0.37)	0.67 (-32.59 to 33.94)	0.968	0.01 (-0.37 to 0.39)
Pallidum	-0.37 (-12.04 to 11.30)	0.950	-0.01 (-0.39 to 0.37)	-0.27 (-12.28 to 11.75)	0.965	-0.01 (-0.39 to 0.37)
Hippocampus	-20.86 (-67.79 to 26.07)	0.381	-0.15 (-0.53 to 0.23)	-19.94 (-68.39 to 28.51)	0.417	-0.14 (-0.52 to 0.24)
Amygdala	-2.98 (-11.60 to 5.65)	0.496	-0.12 (-0.50 to 0.26)	-2.11 (-11.00 to 6.78)	0.639	-0.09 (-0.46 to 0.29)

Legend: Mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes for associations between brain volumes and HIV-exposure accounting for maternal alcohol during pregnancy. Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. †Minimally adjusted models included child age at scan, child sex, intracranial volume, and maternal alcohol during pregnancy. ‡Fully adjusted models included child age and child sex, maternal education, household income, maternal age, and maternal alcohol use. Linear regression models where negative estimates indicate HIV exposure is associated with lower volumes in that region. *Uncorrected p<0·05. § p-values survive multiple comparison correction using the false discovery rate across subcortical regions. Abbreviation: CI = Confidence Interval.

Table S8: Adjusted mean differences in grey matter volumes according to HIV exposure status excluding CMV positive cases

Brain volumes	Mean (SD) volume (mm^3)		Minimally adjusted† coefficient (95% CI)	P-value	Effect size	Fully Adjusted‡ coefficient (95% CI)	P-value	Effect size
	HEU (n=37)	HU (n=72)			Cohen's d (95% CI)			Cohen's d (95% CI)
Global volume								
Total grey matter	234,308 (15517)	238,645 (11836)	-4619 (-8965 to -274)	0.037*	-0.34 (-0.74 to 0.06)	-5477 (-10,029 to -926)	0.019*	-0.40 (-0.80 to -0.00)
Subcortical regions								
Thalamus	3200 (114)	3197 (106)	5.19 (-37.15 to 47.53)	0.809	0.05 (-0.35 to 0.44)	10.32 (-34.00 to 54.65)	0.645	0.10 (-0.30 to 0.49)
Caudate	1785 (149)	1884 (143)	-98.13 (-156.51 to -39.74)	0.001*§	-0.62 (-1.02 to -0.21)	-114.70 (-173.95 to -55.44)	<0.001*§	-0.71 (-1.12 to -0.30)
Putamen	2828 (79)	2826 (84)	-0.42 (-32.70 to 31.86)	0.980	-0.01 (-0.40 to 0.39)	0.20 (-34.06 to 34.46)	0.991	0.00 (-0.39 to 0.40)
Pallidum	709 (32)	710 (29)	-1.44 (-13.16 to 10.28)	0.808	-0.05 (-0.44 to 0.35)	-1.70 (-14.08 to 10.67)	0.785	-0.06 (-0.45 to 0.34)
Hippocampus	1571 (160)	1597 (130)	-20.21 (-67.00 to 26.58)	0.394	-0.14 (-0.54 to 0.25)	-25.94 (-75.28 to 23.40)	0.299	-0.18 (-0.58 to 0.21)
Amygdala	535 (32)	542 (21)	-6.30 (-15.38 to 2.78)	0.172	-0.24 (-0.64 to 0.15)	-6.78 (-16.43 to 2.86)	0.166	-0.26 (-0.66 to 0.13)

Legend: Subcortical volumes (mean of left and right hemispheres), mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes for associations between brain volumes and HIV-exposure excluding CMV positive cases and limiting to those with a negative CMV result (n=109). Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. †Minimally adjusted models included child age at scan, child sex and intracranial volume. ‡Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income, and maternal age. Linear regression models where negative estimates indicate HIV exposure is associated with lower volumes in that region. *Uncorrected $p<0{\cdot}05$. § p-values survive multiple comparison correction using the false discovery rate across subcortical regions. Abbreviations: HEU = HIV-exposed and uninfected; HU = HIV-unexposed; CMV = cytomegalovirus. Abbreviation: CI = Confidence Interval.

Table S9: Impact of maternal HIV disease severity (immunological compromise) on caudate and total grey matter volumes

		Caudate (left)				Caudate (right)				Total grey matter			
	N	**Mean (mm^3)**	**SD (mm^3)**	**Adjusted coefficient† (95% CI)**	**P-value**	**Mean (mm^3)**	**SD (mm^3)**	**Adjusted Coefficient† (95% CI)**	**P-value**	**Mean (mm^3)**	**SD (mm^3)**	**Adjusted Coefficient† (95% CI)**	**P-value**
HU group	106	1862	158	*Reference*	-	1911	176	*Reference*	-	238,676	12,127	*Reference*	-
CD4 >500	12	1814	204	-48.82 (-145.42 to 47.78)	0.319	1820	183	-92.03 (-191.44 to 7.37)	0.069	238,184	17,458	-641 (-7210 to 5929)	0.847
CD4 350-500	8	1816	176	-49.27 (-166.52 to 67.98)	0.407	1820	116	-102.21 (-222.86 to 18.45)	0.096	235,248	14,329	-4872 (-12845 to 3102)	0.229
CD4 <350	13	1805	121	-56.15 (-153.47 to 41.16)	0.256	1794	135	-98.91 (-199.05 to 1.24)	0.053	229,323	13,543	-8271 (-14889 to -1653)	0.015*

Legend: Mean differences (adjusted regression coefficients with 95% CIs) and p-values for associations between brain volumes and maternal CD4 cell count during pregnancy (categorized as CD4>500, 350-500 or <350). †Models were adjusted to include child age at scan, child sex and intracranial volume. Linear regression models where negative estimates indicate CD4 is associated with lower volumes in that region. *p<0·05. Abbreviations: HU = HIV-unexposed. Test for dose-response in total grey matter volumes using the likelihood ratio test, p=0.045. Abbreviation: CI = Confidence Interval.

Table S10: Impact of maternal ART regimen and timing of initiation on caudate and total grey matter volumes in the children who are HIV-exposed and uninfected

		Caudate (left)		Caudate (right)		Total grey matter	
	N	**Adjusted coefficient† (95% CI)**	**P-value**	**Adjusted coefficient† (95% CI)**	**P-value**	**Adjusted coefficient† (95% CI)**	**P-value**
ART alone							
AZT only	5	*Reference*		*Reference*		*Reference*	
1st line triple therapy	34	125.38 (-26.48 to 277.25)	0.103	24.04 (-124.37 to 172.44)	0.744	9632 (-2119 to 21382)	0.105
ART initiation							
Before conception	15	*Reference*		*Reference*		*Reference*	
During pregnancy	25	65.41 (-41.45 to 172.27)	0.222	43.17 (-55.69 to 142.03)	0.381	-1769 (-9917 to 6378)	0.662

Legend: Mean differences (adjusted regression coefficients with 95% CIs) and p-values for associations between brain volumes and maternal ART regimen and timing. ART regimen was categorized into zidovudine (AZT) versus 1st line regimens (the one child born to a mother on a second-line protease inhibitor containing regimen was excluded due to small group sizes). ART timing was dichotomized into initiation before conception versus during pregnancy. †Models were adjusted to include child age at scan, child sex and intracranial volume. Abbreviations: ART = antiretroviral therapy; AZT = zidovudine; CI = Confidence Interval.

Supplementary references

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Appendix IX: Supplementary Information Chapter 9

Subcortical brain volumes and neurocognitive function in children with perinatal HIV exposure: a population-based cohort study in South Africa *(research paper)*

Supplementary Information

Subcortical brain volumes and neurocognitive function in children with perinatal HIV exposure: a population-based cohort study in South Africa

Catherine J Wedderburn, Shunmay Yeung, Nynke A Groenewold, Andrea M Rehman, Sivenesi Subramoney, Jean-Paul Fouche, Shantanu H. Joshi, Katherine L Narr, Nadia Hoffman, Annerine Roos, Diana M Gibb, Heather J Zar, Dan J Stein,* Kirsten A Donald*

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This PDF file includes:

Supplementary Table 1: Post hoc hemispheric analyses of regions with a significant association with HIV exposure

Brain volumes	Unadjusted difference (95% CI) (n=162)	p-value	Effect size Cohen's d (95% CI)	Fully Adjusted † difference (95% CI)	p-value	Effect size Cohen's d (95% CI)
Putamen						
Left hemisphere	-218.70 (-408.29 to -29.10)	0.024*	-0.35 (-0.67 to -0.04)	-188.17 (-361.64 to -8.71)	0.040*	-0.31 (-0.62 to 0.01)
Right hemisphere	-213.26 (-392.69 to -33.82)	0.020*	-0.37 (-0.68 to -0.05)	-174.20 (-339.82 to -8.59)	0.039*	-0.30 (-0.61 to 0.01)
Hippocampus						
Left hemisphere	-110.50 (-211.83 to -9.17)	0.033*	-0.33 (-0.64 to -0.02)	-88.11 (-169.80 to -6.42)	0.035*	-0.26 (-0.58 to 0.05)
Right hemisphere	-101.70 (-214.65 to 11.25)	0.077	-0.28 (-0.59 to 0.04)	-84.56 (-183.30 to 14.18)	0.093	-0.23 (-0.54 to 0.08)

Legend: Mean differences (regression coefficients unadjusted and fully adjusted in multiple regression models), p-values and effect sizes for associations between hemispheric brain volumes and HIV exposure. Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. † Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income, and maternal age. *$p<0.05$

Supplementary Table 2: Associations of maternal CD4 cell count and viral load with subcortical regional volumes

Brain volume	Maternal CD4 (cells/microlitres)	Mean volume (SD) (mm^3)	*N*	Fully adjusted difference (95% CI)	*p*-value
Total subcortical brain volume	HIV-unexposed	48311 (4092)	92	*Reference*	0.065
	≥500	46958 (4211)	28	-718.82 (-1814.66 to 377.02)	
	350-500	46754 (3449)	16	-1162.79 (-2530.19 to 204.62)	
	<350	46380 (6421)	17	-1604.63 (-2959.44 to -249.83)	
Putamen	HIV-unexposed	4597 (543)	92	*Reference*	0.028*
	≥500	4389 (546)	28	-163.26 (-368.82 to 42.29)	
	350-500	4380 (472)	16	-185.22 (-441.71 to 71.27)	
	<350	4207 (706)	17	-362.09 (-616.22 to -107.96)	
Hippocampus	HIV-unexposed	3149 (341)	92	*Reference*	0.060
	≥500	2959 (287)	28	-154.97 (-268.26 to -41.68)	
	350-500	3102 (235)	16	-31.58 (-172.92 to 109.78)	
	<350	3122 (439)	17	-9.68 (-149.74 to 130.37)	
Brain volume	**Maternal viral load, (copies/mL)**	**Mean volume (SD) (mm^3)**	***N***	**Fully adjusted difference (95% CI)**	***p*-value**
Total subcortical brain volume	HIV-unexposed	48311 (4092)	92	*Reference*	0.021*
	<40 (undetectable)	47213 (4762)	44	-632.10 (-1612.91 to 348.71)	
	≥40 (detectable)	46081 (5869)	13	-2248.18 (-3858.37 to -638.00)	
Putamen	HIV-unexposed	4597 (543)	92	*Reference*	0.014*
	<40 (undetectable)	4447 (594)	44	-123.87 (-304.69 to 56.94)	
	≥40 (detectable)	4176 (590)	13	-434.68 (-731.52 to -137.84)	
Hippocampus	HIV-unexposed	3149 (341)	92	*Reference*	0.067
	<40 (undetectable)	3033 (331)	44	-95.78 (-194.59 to 3.04)	
	≥40 (detectable)	3014 (379)	13	-147.97 (-310.19 to 14.25)	

Legend: Total subcortical and regional volumes (mean of left and right hemispheres), mean differences (regression coefficients fully adjusted in multiple regression models), and p-values for associations between brain volumes and maternal CD4 cell count or maternal viral load in pregnancy. Models were adjusted for child age and sex, intracranial volume, household income, maternal age, and education. Regions that had associations with HIV exposure were selected. P-values from the Wald test. *p<0.05.

Supplementary Table 3a: Adjusted mean differences in subcortical brain volumes according to HIV exposure controlling for any breastfeeding

Subcortical brain volumes	Fully Adjusted † difference (95% CI)	P-value	Effect size Cohen's d (95% CI)
Total subcortical volume	-1218.94 (-2153.02 to -284.85)	0.011*	-0.28 (-0.59 to 0.04)
Thalamus	-96.10 (-251.67 to 59.47)	0.224	-0.17 (-0.48 to 0.14)
Caudate	-117.50 (-248.91 to 13.91)	0.079	-0.23 (-0.55 to 0.08)
Putamen	-192.69 (-375.91 to -9.47)	0.039*	-0.34 (-0.65 to -0.02)
Pallidum	-38.81 (-103.79 to 26.16)	0.240	-0.18 (-0.49 to 0.13)
Hippocampus	-122.86 (-225.24 to -20.48)	0.019*	-0.36 (-0.67 to -0.05)
Amygdala	-36.61 (-88.34 to 15.11)	0.164	-0.23 (-0.54 to 0.08)
Nucleus accumbens	10.11 (-19.70 to 39.92)	0.504	0.12 (-0.19 to 0.43)

Legend: Adjusted mean differences, 95% confidence intervals and p-values for associations between brain volumes and HIV exposure accounting for breastfeeding. Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income, and maternal age. *p<0.05. Similar results were seen adjusting for exclusive breastfeeding.

Supplementary Table 3b: Adjusted mean differences in subcortical brain volumes according to HIV exposure controlling for duration of exclusive breastfeeding

Subcortical brain volumes	Fully Adjusted † difference (95% CI)	P-value	Effect size Cohen's d (95% CI)
Total subcortical volume	-1032.04 (-1838.91 to -225.16)	0.013*	-0.24 (-0.55 to 0.08)
Thalamus	-91.61 (-212.61 to 29.38)	0.137	-0.16 (-0.47 to 0.15)
Caudate	-64.55 (191.74 to 62.64)	0.318	-0.13 (-0.44 to 0.18)
Putamen	-183.92 (-342.35 to -25.50)	0.023*	-0.32 (-0.63 to -0.01)
Pallidum	-25.70 (-82.07 to 30.68)	0.369	-0.12 (-0.43 to 0.19)
Hippocampus	-95.30 (-178.76 to -11.83)	0.026*	-0.28 (-0.59 to 0.03)
Amygdala	-32.99 (-78.94 to 12.95)	0.158	-0.20 (-0.52 to 0.11)
Nucleus accumbens	14.60 (-10.88 to 40.07)	0.259	0.17 (-0.14 to 0.48)

Legend: Adjusted mean differences, 95% confidence intervals and p-values for associations between brain volumes and HIV exposure accounting for duration of exclusive breastfeeding. Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income, maternal age and duration of exclusive breastfeeding. *p<0.05. Similar results were seen adjusting for any breastfeeding duration.

Supplementary Table 4: Adjusted mean differences in subcortical brain volumes according to HIV exposure excluding imaging outliers

Brain volumes	Fully Adjusted coefficient (95% CI)	P-value	Effect size Cohen's d (95% CI)
Total volume			
Subcortical grey matter	-876.32 (-1701.85 to -50.78)	0.038*	-0.25 (-0.58 to 0.08)
Subcortical regions			
Thalamus	-66.20 (-191.19 to 58.78)	0.297	-0.13 (-0.46 to 0.20)
Caudate	-55.97 (-194.80 to 82.87)	0.427	-0.12 (-0.45 to 0.21)
Putamen	-169.93 (-320.37 to -19.49)	0.027*	-0.35 (-0.69 to -0.02)
Pallidum	-24.33 (-76.45 to 27.79)	0.358	-0.14 (-0.47 to 0.19)
Hippocampus #	-71.32 (-158.38 to 15.74)	0.108	-0.24 (-0.57 to 0.09)
Amygdala	-30.51 (-73.89 to 12.87)	0.167	-0.22 (-0.55 to 0.11)
Nucleus Accumbens	20.33 (-6.33 to 47.00)	0.134	0.27 (-0.07 to 0.60)

Legend: Adjusted mean differences, 95% confidence intervals and p-values for associations between brain volumes and HIV exposure excluding outliers (n=145). Fully adjusted models included child age at scan, child sex, intracranial volume, maternal education, household income, and maternal age. *p<0.05. #Left hippocampus: -92.04 (-173.80 to -10.28), p=0.028*, effect size -0.32 (-0.65 to 0.01).

Appendix X: Supplementary Information Chapter 10

Association of *in utero* HIV exposure with child brain structure and language development: a South African birth cohort study *(research paper)*

Supplementary Information

Association of *in utero* HIV exposure with child brain structure and language development: a South African birth cohort study

Catherine J Wedderburn, Shunmay Yeung, Sivenesi Subramoney, Jean-Paul Fouche, Shantanu H. Joshi, Katherine L. Narr, Andrea M Rehman, Annerine Roos, Diana M Gibb, Heather J Zar, Dan J Stein, Kirsten A Donald

This PDF file includes:

S1 Information: Detailed methods for image processing and analysis

Image acquisition
A 3D MEMPRAGE (Multi-Echo Magnetization Prepared Rapid Acquisition Gradient Echo) sequence was used in sagittal orientation with repetition time (TR) = 2530ms, echo time (TE) = 1.69, 3.54, 5.39, 7.24 ms, inversion time (TI) = 1100ms, flip angle = 7.0°, voxel size 1.0x1.0x1.0 mm^3, field of view (FOV) = 224x224x176 mm, 176 slices, 1.0 mm thick. The overall duration was 5 minutes 21 seconds.

Image processing
Images were processed with FreeSurfer version 6.0 software (http://surfer.nmr.mgh.harvard.edu/) at the local supercomputing cluster at the Centre for High Performance Computing (CHPC, Cape Town) (https://www.chpc.ac.za). The automated process includes motion correction, skull stripping, Talairach transformation, intensity normalization, volumetric segmentation and surface-based cortical reconstruction to produce anatomical measures [1-5]. The cortex was parcellated into regions of interest (ROIs) according to the Desikan-Killiany atlas [6], and measures of cortical structure (cortical thickness, mm and surface area, mm^2) were extracted for analysis. Cortical thickness represents the shortest distance (mm) between the pial (grey-CSF boundary) and grey-white matter surfaces [2].

Region-of-interest selection
All regions in the prefrontal cortex were selected *a priori* as they were determined to be biologically plausible areas due to their role in cognitive functioning. The prefrontal cortex is vulnerable to early environmental exposures and associations between the prefrontal cortex and neurocognitive development have been noted across ages [7, 8]. Studies of children with HIV suggest frontal regions are affected [9-11]. Given documented neurocognitive delay in children who are HEU [12, 13], we therefore hypothesised that regions in the prefrontal cortex would be affected. We used the automated Desikan-Killiany parcellation [6] of the prefrontal cortex as described in prior papers [14] and included all regions as follows: superior frontal, caudal middle frontal, rostral middle frontal, medial orbitofrontal, lateral orbitofrontal, inferior frontal gyrus (the pars triangularis, pars opercularis and pars orbitalis combined) and frontal pole. For each participant, the mean values of left and right hemispheres were used for subsequent analyses of each measure (cortical thickness, cortical surface area).

Mediation Analyses
We performed a mediation analysis to evaluate whether, and to what extent, the effect of HIV exposure on language is mediated through cortical thickness – a hypothesis generated from findings in the prior analyses. Mediation analysis provides an estimation of the direct effect, indirect effect and total effect of HIV exposure (exposure) on language function (outcome), and whether the indirect effect is mediated by cortical thickness (mediator). We applied the Baron and Kenny (1986) approach that uses sequential regression analyses [15]. The criteria for mediation are that there are associations between: (1) HIV exposure and language development (path *c*); (2) HIV exposure and cortical thickness (path *a*); (3) cortical thickness and language score (path *b*) controlling for the exposure; and that (4) the effect of HIV exposure on language function is lost (full mediation) or reduces (partial mediation) after controlling for cortical thickness (path *c'*). Models were adjusted for potential confounding variables identified *a priori*. The percentage that the mediator contributed was calculated as the ratio of the indirect effect coefficient to the total effect coefficient (proportion of total effect mediated). A reduction of the direct effect coefficient after adjusting for the mediator was taken as evidence of mediation. We confirmed the findings using structural equation modelling.

S1 Table: Comparison of Drakenstein Child Health study demographics of children with imaging versus those without imaging

	DCHS cohort[#]		
	Neuroimaging 162	**No neuroimaging 979**	***P***
Male sex	94 (58.0%)	492 (50.3%)	0.067
Monthly household income (ZAR)			
< R1000 (<~$75)	111 (68.5%)	599 (61.2%)	0.075
>R1000 (>~$75)	51 (31.5%)	380 (38.8%)	
Maternal education			
Secondary	108 (66.7%)	586 (59.9%)	0.100
Completed secondary	54 (33.3%)	393 (40.1%)	
Maternal employment status (employed)	44 (27.2%)	263 (26.9%)	0.937
Birthweight, g	3091 (574)	3015 (607)	0.134
Birth head circumference, cm	33.6 (2.0)	33.5 (2.1)	0.411
Maternal smoking during pregnancy	32 (19.8%)	230 (23.7%)	0.274
Maternal alcohol use during pregnancy	24 (18.2%)	107 (12.4%)	0.067
Exclusive breastfeeding duration, months	1.8 (1.8)	2.1 (2.0)	0.068

Legend: Data are N(%), mean (SD). Continuous variables were compared with unpaired t-tests; categorical variables were compared with Chi-squared tests. #Full DCHS cohort excluding two children with HIV-infection. Percentages are cited among those with non-missing values. Missing data: birthweight (n=13); birth head circumference (n=23); maternal smoking (n=7); maternal alcohol use (n=146); breastfeeding (n=77).

S2 Table: Antiretroviral drug regimens received by mothers with HIV during pregnancy

Antiretroviral drug (ARV) regimen	Child exposure N (%)
AZT monotherapy	1 (1.4%)
1st line three-drug ART (2 NRTIs + NNRTI)	
TDF + (3TC or FTC) + EFV (separately or fixed dose combination)	64 (91.4%)
AZT + 3TC + EFV	1 (1.4%)
TDF + FTC + NVP	1 (1.4%)
AZT + 3TC + NVP	2 (2.9%)
2nd line ART (2 NRTIs + PI)	
AZT + 3TC + LPV/r	1 (1.4%)

Legend: Abbreviations: ART = Antiretroviral therapy; AZT = zidovudine; EFV = efavirenz; 3TC = lamivudine; FTC = emtricitabine; TDF = tenofovir; NVP = nevirapine; LPV/r = lopinavir/ritonavir (Kaletra); NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor

S3 Table: Adjusted mean differences in cortical thickness according to HIV exposure restricted to one site

	Cortical thickness, mean SD)		Minimally adjusted model[a]			Full adjusted model[b]		
Region	HEU (n=65)	HU (n=49)	Mean difference (95% CI)	p-value	Effect size	Mean difference (95% CI)	p-value	Effect size
Superior frontal	3.31 (0.16)	3.28 (0.17)	0.02 (-0.04 to 0.09)	0.471	0.14 (-0.23 to 0.51)	0.03 (-0.03 to 0.10)	0.343	0.19 (-0.18 to 0.56)
Caudal middle frontal	2.96 (0.16)	2.95 (0.15)	0.00 (-0.06 to 0.06)	0.907	0.02 (-0.35 to 0.39)	0.01 (-0.06 to 0.07)	0.845	0.04 (-0.33 to 0.41)
Rostral middle frontal	2.97 (0.12)	2.95 (0.13)	0.00 (-0.04 to 0.05)	0.930	0.02 (-0.35 to 0.39)	0.01 (-0.04 to 0.06)	0.665	0.08 (-0.29 to 0.45)
Medial orbitofrontal	3.22 (0.22)	3.12 (0.18)	0.10 (0.02 to 0.17)	0.011*	0.49 (0.11 to 0.86)	0.10 (0.02 to 0.18)	0.015*	0.49 (0.11 to 0.86)
Lateral orbitofrontal	3.26 (0.14)	3.24 (0.13)	0.02 (-0.03 to 0.08)	0.415	0.16 (-0.21 to 0.53)	0.03 (-0.03 to 0.08)	0.286	0.21 (-0.16 to 0.58)
Inferior frontal	3.14 (0.13)	3.14 (0.14)	-0.00 (-0.06 to 0.05)	0.893	-0.03 (-0.40 to 0.34)	0.01 (-0.05 to 0.06)	0.827	0.04 (-0.33 to 0.41)
Frontal pole	3.52 (0.26)	3.49 (0.27)	0.02 (-0.08 to 0.12)	0.746	0.06 (-0.31 to 0.43)	0.03 (-0.08 to 0.13)	0.589	0.11 (-0.26 to 0.48)

Legend: Multiple linear regression estimates for HIV exposure on cortical thickness (mm) restricted to the clinic site where the majority of HEU children attend. *p<0·05 [a]Adjusted for child age and sex; [b]Adjusted for child age and sex, household income, maternal age and education. Cortical thickness (mean of left and right hemispheres), mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes are presented. Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. Residuals were assessed for each model using quantile-quantile plots and were normally distributed. A positive regression estimate indicates HIV exposure is associated with thicker cortices in that region. Abbreviations: HEU = children who are HIV-exposed and uninfected; HU = children who are HIV-unexposed.

S4 Table: Adjusted mean differences in cortical thickness according to HIV exposure restricting to HEU children born to mothers on the same first-line ART regimen

	Minimally adjusted model[a]			Full adjusted model[b]		
Region	Mean difference (95% CI)	p-value	Effect size	Mean difference (95% CI)	p-value	Effect size
Superior frontal	0.01 (-0.04 to 0.07)	0.592	0.09 (-0.23 to 0.41)	0.03 (-0.03 to 0.08)	0.311	0.17 (-0.15 to 0.49)
Caudal middle frontal	0.02 (-0.03 to 0.07)	0.457	0.12 (-0.19 to 0.44)	0.02 (-0.03 to 0.08)	0.397	0.15 (-0·17 to 0.47)
Rostral middle frontal	0.02 (-0.02 to 0.06)	0.281	0.18 (-0.14 to 0.50)	0.04 (-0.00 to 0.08)	0.081	0.30 (-0·02 to 0.62)
Medial orbitofrontal	0.08 (0.01 to 0.14)	0.020*	0.39 (0.06 to 0.71)	0.09 (0.02 to 0.15)	0.011*	0.44 (0·12 to 0.76)
Lateral orbitofrontal	0.02 (-0.03 to 0.07)	0.426	0.13 (-0.19 to 0.45)	0.03 (-0.01 to 0.08)	0.163	0.24 (-0.08 to 0.56)
Inferior frontal	0.00 (-0.04 to 0.05)	0.854	0.03 (-0.29 to 0.35)	0.02 (-0.02 to 0.07)	0.356	0.16 (-0.16 to 0.48)
Frontal pole	0.04 (-0.05 to 0.14)	0.368	0.15 (-0.17 to 0.47)	0.06 (-0.03 to 0.16)	0.196	0.23 (-0.09 to 0.55)

Legend: Multiple linear regression estimates for HIV exposure on cortical thickness (mm) restricting HEU to children born to mothers on the same WHO recommended first-line ART regimen (efavirenz + emtricitabine/lamivudine + tenofovir) (n=64); total n=156. *p<0.05 [a]Adjusted for child age and sex; [b]Adjusted for child age and sex, household income, maternal age and education. Cortical thickness (mean of left and right hemispheres), mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes are presented. Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. A positive regression estimate indicates HIV exposure is associated with thicker cortices in that region. Abbreviations: HEU = children who are HIV-exposed and uninfected.

S5 Table: Comparison of cognitive, language, and motor development between HEU and HU children

BSID-III domain (composite scores)	Composite Score Mean (SD)		Minimally adjusted model[a]			Fully adjusted model[b]		
	HEU	HU	Mean difference	p-value	Effect size	Mean difference	p-value	Effect size
Cognition	85.00 (9.30)	87.44 (9.29)	-2.36 (-5.48 to 0.77)	0.138	-0.25 (-0.58 to 0.08)	-1.98 (-5.22 to 1.26)	0.228	-0.21 (-0.54 to 0.12)
Language	81.82 (10·68)	86.25 (11.84)	-4.52 (-8.43 to -0.62)	0.024*	-0.38 (-0.73 to -0.04)	-5.16 (-9.13 to -1.18)	0.011*	-0.44 (-0.78 to -0.09)
Motor	93.22 (11.38)	94.47 (10.55)	-1.44 (-5.17 to 2.29)	0.446	-0.13 (-0.47 to 0.20)	-1.03 (-4.93 to 2.87)	0.602	-0.09 (-0.43 to 0.24)

BSID-III domain (raw Scores)	Raw Score Mean (SD)		Minimally adjusted model[a]			Fully adjusted model[b]		
	HEU	HU	Mean difference	p-value	Effect size	Mean difference	p-value	Effect size
Cognition	55.38 (4.97)	56.64 (4.61)	-1.27 (-2.87 to 0.32)	0.116	-0.26 (-0.60 to 0.07)	-1.14 (-2.79 to 0.512)	0.175	-0.24 (-0.57 to 0.09)
Receptive language	19.93 (3.07)	21.28 (3.76)	-1.36 (-2.51 to -0.21)	0.021*	-0.38 (-0.71 to -0.04)	-1.57 (-2.75 to -0.39)	0.010*	-0.43 (-0.77 to -0.10)
Expressive language	23.23 (5.69)	25.00 (5.26)	-1.90 (-3.74 to -0.07)	0.042*	-0.34 (-0.68 to -0.00)	-2.22 (-4.05 to -0.38)	0.018*	-0.40 (-0.74 to -0.05)
Fine motor	37.57 (3.22)	37.91 (3.28)	-0.37 (-1.44 to 0.71)	0.500	-0.11 (-0.44 to 0.22)	-0.23 (-1.34 to 0.88)	0.688	-0.07 (-0.40 to 0.26)
Gross motor	53.72 (2.89)	53.67 (3.09)	-0.05 (-1.07 to 0.76)	0.926	-0.02 (-0.35 to 0.32)	-0.02 (-1.10 to 1.06)	0.968	-0.01 (-0.34 to 0.33)

Legend: Composite neurodevelopmental domain scores, mean differences (regression coefficients minimally and fully adjusted in multiple regression models), p-values and effect sizes for associations between neurodevelopment and HIV exposure. Supplementary table with raw scores below. [a]Adjusted for child age and sex; [b]Adjusted for child age and sex, household income, maternal age and education. *p<0·05. Effect sizes were calculated using Cohen's d with associated 95% confidence intervals. Residuals were assessed for each model using quantile-quantile plots and were normally distributed. A negative regression estimate indicates HIV exposure is associated with lower scores in that domain. Cognitive n=146 (HEU 60, HU 86); Language n=138 (HEU 57, HU 81); Motor n=141 (HEU 58, HU 83). Abbreviations: HEU = children who are HIV-exposed and uninfected; HU = children who are HIV-unexposed.

S6 Table: Correlations between cortical thickness and language development stratified by HIV exposure

	Pearson's correlations					
	Total		HEU		HU	
Region	***r***	***p***	***r***	***p***	***r***	***p***
Superior frontal	-0.17	0.041*	-0.25	0.057	-0.10	0.388
Caudal middle frontal	-0.17	0.046*	-0.16	0.242	-0.14	0.223
Rostral middle frontal	-0.19	0.028*	-0.18	0.175	-0.14	0.211
Medial orbitofrontal	-0.31	0.0002*	-0.35	0.008*	-0.23	0.038*
Lateral orbitofrontal	-0.14	0.112	-0.14	0.312	-0.11	0.317
Inferior frontal	-0.18	0.031*	-0.14	0.305	-0.19	0.084
Frontal pole	-0.08	0.366	0.03	0.821	-0.11	0.344

Legend: Pearson's correlations between language development and HIV exposure. *p<0·05. Total n=138 (HEU 57; HU 81). Abbreviations: HEU = children who are HIV-exposed and uninfected; HU = children who are HIV-unexposed; mOFC = medial orbitofrontal cortex.

S7 Table: Structural equation model

Variable (n=138)	
Direct effects	**β, 95% CI, *p*-value**
HIV exposure → mOFC thickness *(path a)*	0.52 (0.18 to 0.86), p=0.003
mOFC thickness → Language *(path b)*	-0.23 (-0.39 to -0.07), p=0.004
HIV exposure → Language *(path c)*	-0.23 (-0.56 to 0.10), p=0.177
Indirect effects	
HIV exposure → Language *(ab)*	-0.12 (-0.23 to -0.01), p=0.038
Total effects	
HIV exposure → mOFC thickness	0.52 (0.18 to 0.86), p=0.003
mOFC thickness → Language	-0.23 (-0.39 to -0.07), p=0.004
HIV exposure → Language *(ab + c)*	-0.35 (-0.68 to -0.02), p=0.039

*Covariates included in models: child age and sex, household income, maternal age and education

Proportion of total effect mediated: indirect/total effect: 0.35
Ratio of indirect to direct effect: indirect/direct: 0.53
Ratio of total to direct effect: total/direct: 1.53

Summary:[16] Estimates of the direct and indirect (mediated through brain structure) effect of HIV exposure on language development, measured by the BSID-III are shown. All scores were standardized. The total effect for HIV exposure on language development is -0.35. The direct effect for HIV exposure on language development is smaller than the total effect (-0.23). The indirect effect of HIV that passes through the mediator, mOFC thickness, is -0.12, which is statistically significant. The proportion of the total effect that is mediated is approximately one third (35%), and the ratio of the indirect effect:direct effect is approximately half the size of the direct effect (0.53). Similar results are obtained in unadjusted analyses, holding on bootstrapping with 1000 repetitions. Significance testing of indirect effect (adjusted, standardized) using the Sobel method showed mediation is complete (p=0.032). Similar results were obtained using the Monte Carlo test. Abbreviation: mOFC: medial orbitofrontal cortex

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Appendix XI: Language outcomes of preschool children who are HIV-exposed and uninfected: an analysis of a South African cohort

(last author research paper)

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PLOS ONE

RESEARCH ARTICLE

Language outcomes of preschool children who are HIV-exposed uninfected: An analysis of a South African cohort

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Abstract

Introduction

There are approximately 16 million children who are HIV-exposed and uninfected (CHEU) worldwide. Studies suggest that CHEU are at risk for developmental impairment in infancy, particularly in language domains. However, there is limited research examining neurocognitive function in CHEU older than 2 years, including important pre-school years. This study aimed to investigate associations between HIV exposure without infection and neurocognitive outcomes and to determine risk factors for neurodevelopment in CHEU at age 3–4 years.

Methods

The Drakenstein Child Health Study is a South African population-based birth cohort which enrolled women in pregnancy with ongoing follow up. Neurocognitive outcomes were assessed in children at 3.5 years by trained assessors blinded to HIV status including general cognitive function, language, and memory, measured using the Kaufmann Assessment Battery for Children, Second Edition (KABC-II). Data were compared between CHEU and children who were HIV-unexposed uninfected (CHUU) using multivariable logistic and linear regression, including testing for effect modification; sex-stratified risk factor analyses were performed.

nominated investigators approved by the University of Cape Town Human Research Ethics Committee, as per the consent document. Interested, qualified researchers may request to access this data by contacting the Drakenstein Child Health Study (via Lesley Workman, Senior Data Manager at lesley.workman@uct.ac.za) to submit a formal data use request and ensure required ethical approval received prior to use.

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Results

A total of 497 children were included (97 [20%] CHEU; 400 [80%] CHUU; 50% male), with a mean age of 3.5 years (range 3.4–3.6). Groups had similar birth and household characteristics, although mothers of CHEU were older, on average. Overall, CHEU had lower expressive language scores compared to CHUU on unadjusted and adjusted analyses (effect size: -0.23 [95% CI -0.45, -0.01]). There were no group differences in general cognitive or memory function (p>0.05). On sex-stratified analyses, male CHEU were found to have higher odds of suboptimal cognitive development compared to male CHUU (aOR 2.28 [95% CI 1.06, 4.87], p = 0.034). Several other factors including birthweight, maternal education, maternal ART duration and HIV viral load during pregnancy were associated with cognition, memory, or expressive language outcomes in CHEU, dependent on child sex.

Interpretation

The findings suggest that perinatal HIV exposure continues to be associated with impaired language development across the preschool years, highlighting the importance of targeting early interventions to optimise language outcomes. Further, the results suggest the importance of demographic, biological and HIV-related variables influencing developmental outcomes in CHEU. The greater risk of suboptimal cognitive development in male CHEU requires investigation around sex-specific mechanisms.

Introduction

The number of people living with HIV remains high, estimated at 39 million globally [1]. Adolescent girls and young women remain disproportionately affected by HIV, accounting for 60% of new HIV infections in that age group [2]. In 2022 sub-Saharan Africa, adolescent girls and young women accounted for over 77% of new infections among 15–24 years olds [3]. However, over the past two decades there has been huge success in preventing vertical transmission of HIV with the scale up of antiretroviral therapy (ART) during pregnancy. Therefore, most children born to women living with HIV are HIV-exposed but remain uninfected; this population is estimated at 16 million worldwide and represents over 20% of children born in some countries, including South Africa [1].

Children who are HIV-exposed and uninfected (CHEU) are increasingly recognised as being vulnerable to poor health outcomes [4]. Through the Sustainable Development Goals there has been a shift to focus on ensuring that children not only survive, but also thrive. Previous reviews have found that CHEU have lower scores across many neurocognitive domains when compared with HIV-unexposed uninfected children (CHUU) [5, 6], and language has been noted to be a specific area of risk [7]. A recent meta-analysis of eight large studies of child neurodevelopment found that CHEU have particular risk of deficits in expressive language and gross motor function compared to CHUU by 24 months [8]. However, meta-analysis was not possible at older ages due to the scarcity of studies.

There is limited available research on cognition in CHEU older than 24 months [5]. One study in Malawi and Uganda using the Kaufman Assessment Battery for Children, 2nd edition (KABC-II) reported similar cognitive outcomes between CHEU and CHUU from infancy up to five years [9]. Comparable findings were seen in school-age children [10]. In Cameroon, authors found differences in KABC-II composite scores in school-aged CHEU and CHUU, although these differences were no longer apparent after adjusting for contextual factors [11].

However, only a few studies have included language outcomes in this age range. Researchers from the Democratic Republic of Congo reported differences between language impairment in CHEU compared to CHUU at 18–72 months [12]. Separately, a study of 2–12 year olds in Cambodia and Thailand also found poorer verbal ability, along with lower IQ, in CHEU [13]. However, many of these studies were conducted prior to the roll out of ART for all pregnant women, and differences in populations and study quality mean that further work is needed to understand neurocognitive outcomes in pre-school and school-age CHEU.

Several risk factors for poor neurocognitive development are highlighted in the literature and may impact upon the HIV exposure-outcome relationship [14]. Prior studies have demonstrated the importance of understanding these contextual factors [15] and having an adequately matched group of unexposed children for comparison [8]. The Drakenstein Child Health Study (DCHS) is a multidisciplinary South African population-based birth cohort, which was established to investigate early-life determinants of child health in a low socioeconomic peri-urban area of the Western Cape [16–18]. This provides a unique opportunity to investigate developmental outcomes on a larger sample than other recent studies in this context, providing access to comprehensive datasets. Previous research in this cohort found a high prevalence of developmental delay [14], and further, that CHEU had poorer receptive and expressive language outcomes at 24 months compared to CHUU [8]. Therefore, this analysis aimed to build upon these findings by assessing neurocognitive outcomes at 3.5 years comparing CHEU with CHUU from the DCHS and investigating the contextual predictors of neurodevelopment in CHEU.

Methods

Study design and setting

The study cohort is part of the DCHS. Pregnant women were recruited to the DCHS between 5 March 2012 and 31 March 2015 at 20–28 weeks' gestation at routine antenatal visits in two public healthcare clinics; mother-child dyads are being followed up in the DCHS with regular visits in addition to routine care. There is little emigration or immigration from or to the community, with strong retention in study follow up [19]. Antenatal HIV prevalence within this cohort was 21%, however, only two children acquired HIV infection from vertical transmission [20]. Inclusion criteria were 1) over 18 years of age, 2) receiving antenatal care at one of the participating clinics and 3) confirmed to be staying in the area for at least the next year. Written informed consent was obtained from all mothers with ongoing follow up of mothers and children. At 3.5 years neurocognitive assessments were conducted of all available children. This study was approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee.

HIV exposure definition

Exposure, maternal HIV status, was confirmed through routine testing as per Western Cape prevention of mother-to-child transmission (PMTCT) guidelines on enrolment to the study. HIV-exposed children received HIV testing at 6 weeks, 9 months, 18 months and post-cessation of breastfeeding. CHEU were defined as those children born to mothers living with HIV but who remained HIV-negative, whereas CHUU children were born to HIV-negative mothers. Over the course of enrolment, provincial PMTCT guidelines changed, so triple ART initiation was dependent on initial CD4 count and clinical status for mothers enrolled before 2013, and mothers who did not fulfil the criteria received zidovudine prophylaxis; if enrolment was 2013 or later there was universal triple ART access. All HIV-exposed children received

prophylaxis at birth. CD4 and viral load counts were accessed from folder reviews and the National Health Laboratory Service. Children diagnosed with HIV were excluded from this analysis.

Outcome definition (neurocognitive function)

Measurement. Neurocognitive function was measured using the KABC-II [21, 22]. The KABC is validated tool used globally to assess cognitive function in children from 3–18 years and has been used in children with HIV infection [23] and validated in South Africa [24, 25]. The tool was developed from neuropsychological theory and focuses on the processes needed to solve problems rather than their content. At 3.5 years, a full neurocognitive assessment was performed on all children available in the cohort by trained research assistants with the aid of interpreters where necessary. The KABC-II instructions were forward and back translated into local languages and consensus of the translations were done with community members to ensure that the translations were appropriate. The test administrators were all trained psychologists with MA level degrees. All data were quality controlled and checked, and missing data were investigated by test administrators.

Domains. The outcomes at 3.5 years are listed in S1 Table. General cognitive function, language, and memory domains were carefully selected to cover critical areas of child development. General cognitive function was measured using the four subtests listed in S1 Table. Raw scores for each test were converted to age-dependent scaled scores using the KABC-II handbook [22]. Scaled scores were then summed and converted to an age-dependent standard nonverbal index score (NVI) according to the KABC-II manual. Scores are standardised using normative data derived from a reference population to have a mean of 100 and a standard deviation of 15. An NVI of greater than one standard deviation or more below the mean is defined as suboptimal cognitive development [22].

Expressive language was measured using the Expressive Vocabulary subtest in the KABC-II. Memory was assessed using the Atlantis subtest within the KABC-II. Neither of the scores derived from the language or memory subtests can be dichotomised as the KABC-II recommend this only for global index scores such as NVI, so these outcomes are continuous, however, they were age-standardised.

Demographic measures

Comprehensive data were collected using adapted questionnaires from the South African Stress and Health study at an antenatal home visit by trained study staff [26]. Demographic data were also collected at postnatal visits. Maternal psychosocial data were collected during the same antenatal visit. Depression was assessed using the Edinburgh Postnatal Depression Scale (EPDS) with a cut-off score of 13 or above. Alcohol use was assessed by the Alcohol, Smoking and Substance Involvement Screening Test, and smoking status using antenatal urine cotinine levels. Birth outcomes, such as birthweight and gestation were collected at delivery. Gestational age at delivery was assessed based on the second trimester ultrasound scan report; where these were not available, maternal report of last menstrual period dates and fundal height measurements were used. Low birthweight was defined as a birthweight of <2500g and prematurity as a gestation period of <37 weeks. Exclusive breastfeeding is recommended during the first six months of life, and we used this definition in this analysis.

Statistical analysis

A complete-case analysis of mother-child pairs was performed, including children who completed all three outcome assessments and had non-missing values for *a priori* covariates.

Maternal and sociodemographic factors for the analytic cohort were expressed as frequencies and percentages for categorical data and means and standard deviations for continuous data. The summary statistics were shown for the total sample and across the two levels of exposure. Comparisons between CHEU and CHUU were made using two sample t-tests for continuous data and Fisher's exact tests and chi for categorical data respectively. Missing data were summarised for these factors, with their distribution across the exposure. A similar comparison was made between those children in the analytic cohort and the full cohort (n = 1141), to examine the distribution of covariates and assess for selection bias. A sensitivity analysis was also performed including all children who completed each assessment.

In order to assess the association between HIV exposure and neurocognitive development, potential confounders were identified by a directed acyclic graph (DAG) developed from the literature [7, 27–29] (S1 Fig). The minimal sufficient adjusted model indicated by the DAG for estimating the total effect of HIV exposure on neurocognitive development included child sex, maternal age at birth, maternal education and socioeconomic status. Linear regression was used to calculate mean difference estimates for the continuous outcomes, general cognitive function, expressive language and memory. General cognitive function was also dichotomised, and a logistic regression model was used to obtain estimates of association, odds ratios. Crude and adjusted odds ratios, their 95% confidence intervals and p-values were presented for group comparisons of general suboptimal cognitive development. Crude and adjusted mean differences, their 95% confidence intervals and p-values, and Cohen's d effect sizes were reported for cognitive function, language and memory; effect sizes were presented as a forest plot.

Effect modification was tested for each covariate remaining in the models using the likelihood ratio test. If a significant interaction was found using a likelihood ratio test, stratum-specific odds ratios/mean differences were presented in the results.

Finally, exploratory risk factor analyses were also performed on the variables identified by the DAG which may lie on the causal pathway. The associations of socioenvironmental variables, maternal psychological factors, birth outcomes, exclusive breastfeeding and HIV-related variables with the neurocognitive outcomes of CHEU were calculated, stratified by child sex given literature suggesting the role this plays in neurodevelopment [14, 29]. All analyses were performed using Stata version 15.1.

Results

Descriptive statistics

A total of 497 mother-child dyads were included in this analysis, 97 (19.5%) CHEU and 400 (80.5%) CHUU. Fig 1 shows a flow diagram of cohort enrolment and sample flow. Table 1 displays the characteristics within the analytic cohort, disaggregated by HIV exposure status. Mean group ages were 3.5 years across both groups (range 3.4–3.6). The groups were comparable in terms of socioeconomic factors, child sex and birth outcomes (birthweight and gestation). However, mothers with HIV tended to be older compared to mothers without HIV (mean 30.4 vs 26.4 years, $p<0.001$) and were less likely to have used alcohol during pregnancy (4.1% vs 14.3% p = 0.006). Data distributions were similar across the analytic cohort and the full cohort (S2 Table).

General cognitive function

General cognitive scores were similar between CHEU (75.77) and CHUU (76.55), adjusted mean difference -0.72 (-4.06, 2.62) p = 0.672, effect size -0.04 (95% -0.27, 0.17). Similar

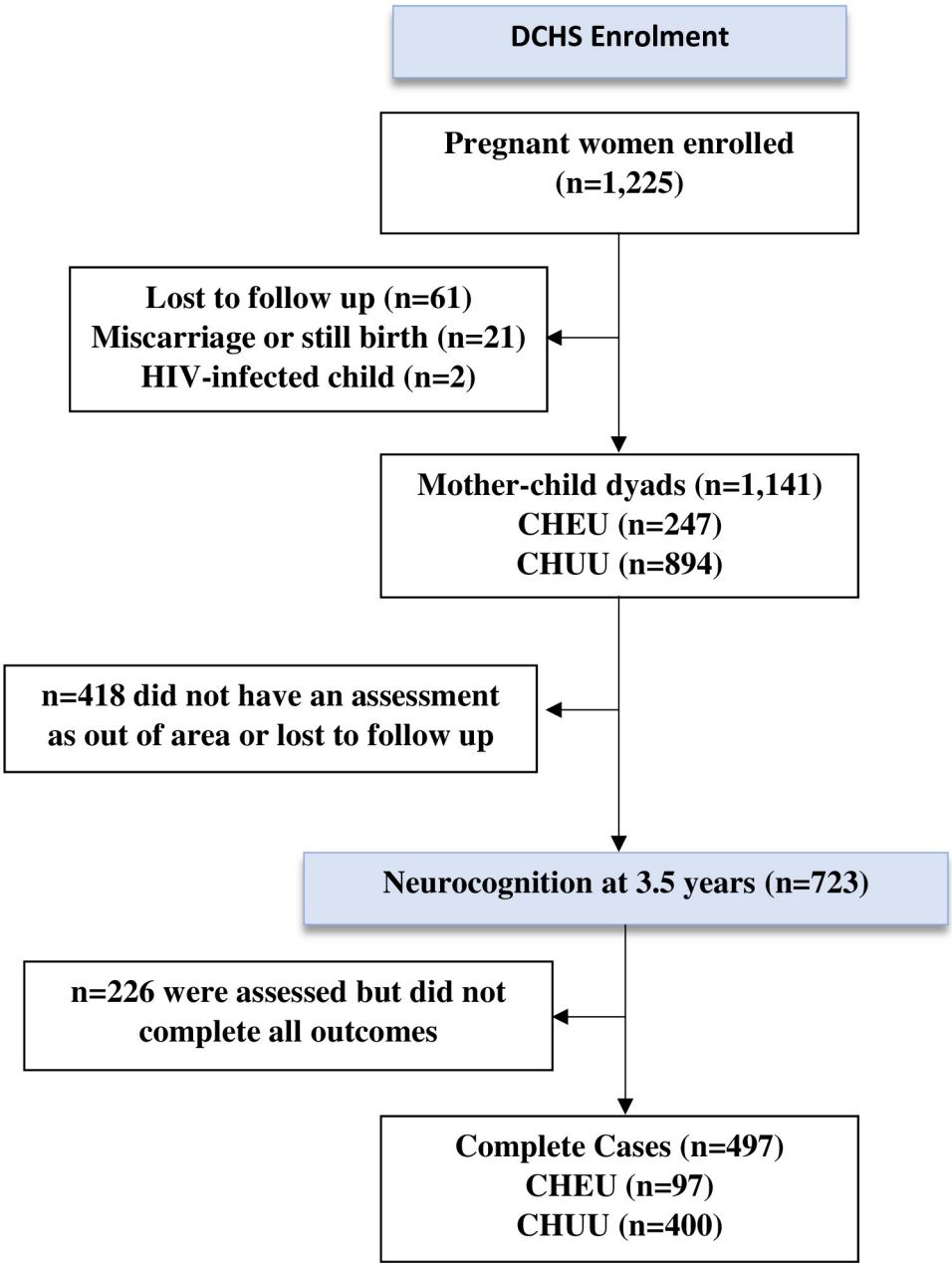

Fig 1. DCHS cohort enrolment and sample used in this analysis.

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proportions of suboptimal cognitive development were also seen on both unadjusted and adjusted analyses (adjusted odds ratio 1.20 [95% CI 0.73, 1.99], p = 0.474) (Fig 2; S3 Table).

Expressive language

On unadjusted analysis, there was an association between HIV exposure and lower expressive language scores (Fig 2; S3 Table). On average, CHEU scored 6.73 (SD = 1.79) and CHUU scored 7.23 (SD = 2.03), with a mean difference of -0.50 (95% CI -0.94, -0.06, p = 0.026), effect size -0.25 (95% CI -0.47, -0.03). The mean difference held on adjusting for covariates (sex,

Table 1. Maternal, child and household characteristics, by HIV exposure.

Variable	Total cohort (n = 497)	CHEU, N (%) (n = 97)	CHUU, N (%) (n = 400)	P-value
Child Characteristics				
Male	249 (50.1)	52 (53.6)	197 (49.3)	0.441
Female	248 (49.9)	45 (46.4)	203 (50.8)	
Birthweight (g), Mean (SD)	3053.7 (579.3)	3040.5 (618.5)	3056.9 (570.3)	0.803
Premature Birth	65 (13.1)	12 (12.4)	53 (13.3)	0.760
Maternal Characteristics				
Maternal age at birth (yrs), Mean (SD)	27.16 (5.69)	30.43 (5.30)	26.36 (5.49)	<0.001*
Education				
Primary	34 (6.8)	7 (7.2)	27 (6.8)	0.072
Some Secondary	268 (53.9)	63 (65.0)	205 (51.2)	
Completed Secondary	166 (33.4)	24 (24.7)	142 (35.5)	
Any Tertiary	29 (5.8)	3 (3.1)	26 (6.5)	
Employed	128 (25.8)	27 (27.8)	101 (25.2)	0.941
Exclusive Breastfeeding for 6 months	81 (16.3)	9 (9.3)	72 (18.0)	0.098
Alcohol use in pregnancy	61 (12.3)	4 (4.1)	57 (14.3)	0.006*
Maternal depression (EPDS threshold)	99 (20.0)	19 (19.6)	80 (20.0)	0.139
Smoker status				
Non-smoker	111 (22.3)	26 (26.8)	85 (21.3)	0.202
Passive smoker	212 (42.7)	45 (46.4)	167 (41.8)	
Active smoker	162 (32.6)	23 (23.7)	139 (34.7)	
Maternal death in first 3.5 years	5 (1.0)	2 (2.1)	3 (0.8)	0.252
Maternal ART regimen (CHEU only)				
PMTCT AZT monotherapy	-	9 (9.3)	-	N/A
First-line triple therapy	-	79 (81.4)	-	
Second-line therapy	-	8 (1.03)	-	
Maternal ART initiation				
Pre-pregnancy	-	39 (40.2)	-	N/A
During pregnancy	-	58 (59.8)	-	
Maternal CD4 count in pregnancy, Mean (SD)	-	501.9 (238.1)	-	N/A
Maternal viral load in pregnancy				
≤ 1000 copies/ml	-	61 (62.9)		N/A
>1000 copies /ml	-	7 (7.2)		
Household Characteristics				
Household income				
<R1000/m	206 (41.2)	41 (42.3)	165 (41.3)	0.921
R1000-R5000/m	234 (47.1)	46 (47.4)	188 (47.0)	
>R5000/m	57 (11.8)	10 (10.3)	47 (11.7)	
Water	346 (69.6)	66 (68.0)	280 (70.0)	0.814
Toilet	315 (63.4)	56 (57.7)	259 (64.8)	0.198

N (%) for categorical variables, mean (SD) for continuous variables.

*p<0.05.

P-values generated using chi-squared test for categorical variables and t-tests or Fisher's exact for continuous variables. Percentages calculated out of all data. Missing data: prematurity n = 2, breastfeeding n = 1, alcohol use n = 50, EPDS n = 50, smoking n = 12, water n = 1. In CHEU only variables, ART regimen n = 1, viral load n = 29, CD4 count n = 12, Abbreviations: CHEU: children who are HIV-exposed uninfected, CHUU: children who are HIV-unexposed uninfected; AZT: zidovudine

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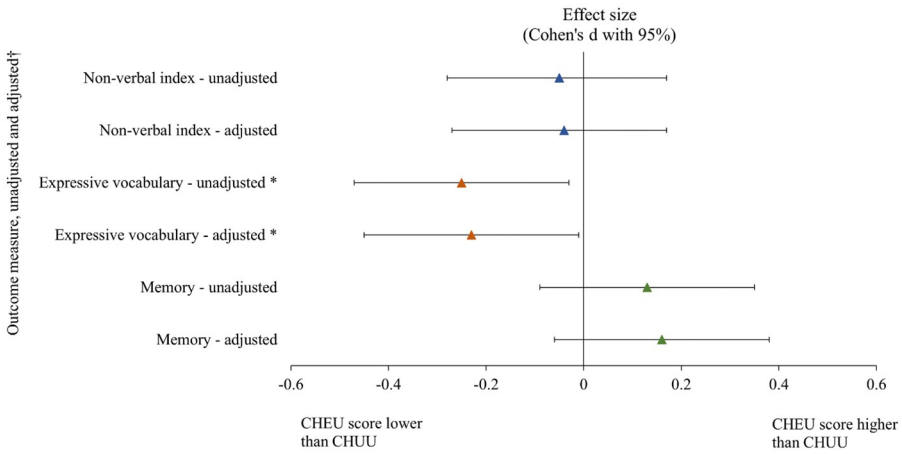

Fig 2. Neurocognitive outcomes at 3.5 years compared between CHEU and CHUU. *p<0.05. † Adjusted for child sex, maternal education, maternal age and household income. *p<0.05. CHEU: Children HIV-exposed uninfected, CHUU: Children HIV-unexposed uninfected.

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maternal age at birth, maternal education and socioeconomic status) (-0.46 [95% CI -0.91, -0.02] p = 0.049) with an effect size of -0.23 (-0.45 to -0.01).

Memory

There were no identified associations between HIV exposure and memory scores on unadjusted (p = 0.257) or adjusted analyses (p = 0.184), with an adjusted mean difference of 0.29 (95% CI -0.14, 0.71) and effect size 0.16 (95% CI -0.06, 0.38) (Fig 2; S3 Table).

A sensitivity analysis of each individual assessment including all children in the cohort who completed each test showed the CHEU/CHUU relationships held (S4 Table).

Potential risk and protective factors

Likelihood ratio tests investigating effect modification on HIV exposure indicated an interaction between HIV exposure and the sex of the child (p = 0.008) for general suboptimal cognitive development. Examining each stratum for sex (Table 2), male CHEU had two times the (adjusted) odds of general suboptimal cognitive development compared to male CHUU (odds ratio of suboptimal cognitive development 2.28 (95% CI 1.06, 4.87, p = 0.034); there were no differences seen between female CHEU and CHUU (p = 0.239). There was a trend for a similar pattern examining cognitive continuous scores, but this was not significant (-3.35 [95% CI -8.06, 1.35]), and no evidence for effect modification in other development domains was detected using likelihood ratio tests.

An analysis of factors that may affect CHEU neurodevelopment was informed by the DAG. Fig 3 shows the results of the risk factor analyses stratified by sex. Overall, we found that higher maternal education (completion of secondary level) was associated with better memory in male CHEU, and better expressive language in female CHEU. Secondly, we found that higher birthweight was associated with improved cognitive function in male CHEU. Other factors, including household income, exclusive breastfeeding, maternal depression in pregnancy and prematurity, did not show evidence of an association with general cognitive function, language, or memory in CHEU in this analytic cohort. In terms of HIV-related variables, we found that initiation of ART before pregnancy (versus during pregnancy) was associated with

Table 2. Sex-stratified unadjusted and adjusted analyses of neurocognitive outcomes at 3.5 years.

General Cognitive Function (NVI suboptimal development)				
Sex/Exposure	**Unadjusted odds ratio (95% CI)**	**p-value**	**Adjusted† odds ratio (95% CI)**	**p-value**
Female				
CHUU	1	0.329	1	0.239
CHEU	0.72 (0.37, 1.39)		0.65 (0.32, 1.33)	
Male				
CHUU	1	0.072	1	0.034*
CHEU	1.88 (0.94, 3.76)		2.28 (1.06, 4.87)	
General Cognitive Function (NVI Score)				
Sex/Exposure	**Unadjusted mean difference (95% CI)**	**p-value**	**Adjusted† mean difference (95% CI)**	**p-value**
Female				
CHUU	Reference	0.691	Reference	0.454
CHEU	0.99 (-3.89, 5.86)		1.82 (-2.97, 6.61)	
Male				
CHUU	Reference	0.286	Reference	0.162
CHEU	-2.37 (-6.73, 1.99)		-3.35 (-8.06, 1.35)	
Expressive language				
Female				
CHUU	Reference	0.147	Reference	0.227
CHEU	-0.44 (-1.04, 0.16)		-0.37 (-0.98, 0.23)	
Male				
CHUU	Reference	0.112	Reference	0.130
CHEU	-0.52 (-1.17, 0.12)		-0.54 (-1.23, 0.16)	
Memory				
Female				
CHUU	Reference	0.766	Reference	0.718
CHEU	0.09 (-0.52, 0.71)		0.12 (-0.52, 0.75)	
Male				
CHUU	Reference	0.195	Reference	0.120
CHEU	0.35 (-0.18, 0.89)		0.46 (-0.12, 1.03)	

Stratum-specific odds presented due to effect modification between sex and HIV (p = 0.008)

† Adjusted for child sex, maternal education, maternal age and household income.

*$p<0.05$.

CHEU: Children HIV-exposed uninfected, CHUU: Children HIV-unexposed uninfected, NVI–Nonverbal Index

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better expressive language in male CHEU, indicating greater ART duration was protective. Further, higher viral load (>1000 copies/ml versus <1000 copies/ml) in mothers during pregnancy was associated with worse cognitive function in female CHEU (Fig 3).

Discussion

The findings of this cohort study indicate that children who are exposed to HIV but remain uninfected have poorer expressive language outcomes at 3–4 years, alongside similar cognitive and memory development, when compared with HIV-unexposed children. The analysis of general cognitive function suggested an interaction between sex and HIV exposure, and that male CHEU are at highest risk for suboptimal cognitive development. Finally, a sex-stratified analysis of risk factors in CHEU found that demographic (maternal education), biological

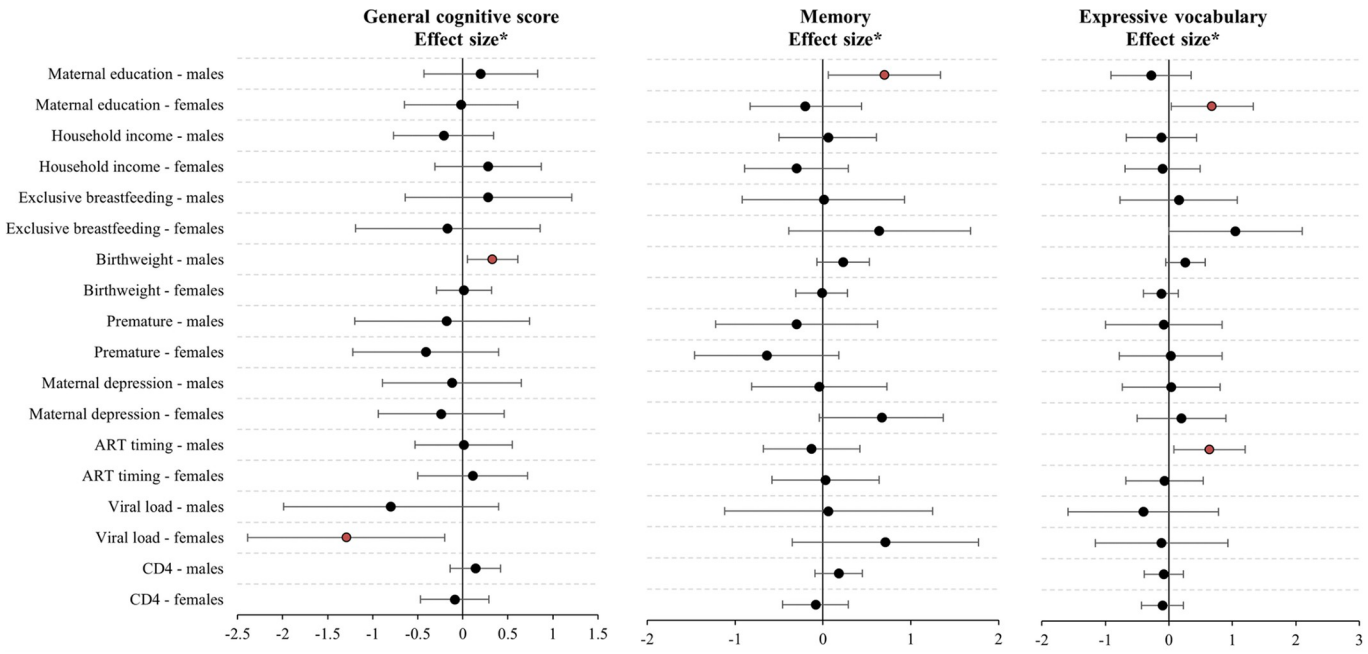

Fig 3. Associations between risk and protective factors with neurocognitive development of CHEU. Red colour indicates p<0.05. * Effect sizes for binary outcomes generated using Cohen's d. For continuous variables (birthweight and CD4 count), regression β values were generated. Factor information: Maternal education compares higher attainment (completed secondary/tertiary) versus primary/some secondary education; Household income compared higher income (>1000 rand/month) versus lower (<1000 rand /month); ART timing compared longer maternal ART (initiation pre-pregnancy versus during pregnancy); Viral load compared unsuppressed viral load (>1000 copies/ml) versus suppressed (<1000 copies/ml).

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(birthweight) and HIV-related factors (maternal ART duration and viral load during pregnancy) were associated with CHEU neurocognitive outcomes, dependent on child sex.

The association between HIV exposure and poor expressive language development builds upon a previous analysis of the cohort at 24 months which found significant differences in language, with a very similar effect size (Cohen's d -0.23 [95%CI -0.41, -0.05]) [7]. Further, it supports a recent meta-analysis including eight studies which detected poorer scores in CHEU on expressive language compared to CHUU by 24 months [8]. The early years of life are a critical period for language development, and these findings suggest that early impairment, although subtle, persists during this time. Neurodevelopment affected by HIV exposure may show differences in language, either as this domain is most affected, or else differences are evident earlier than other domains. The complexity of language development may also mean that this domain is most affected in younger years. Language is known to be important for later school and academic outcomes [30], and there has been a report of ongoing language impairment in youth with HIV exposure [31]. These results suggest the importance of highlighting CHEU as a vulnerable group, detecting any delay in preschool language ability early, and informing the development of interventions to optimise later outcomes.

There was no association between HIV exposure and general cognitive function which is comparable to much of the recent literature, including a large study by Boivin *et al* implementing the KABC-II assessment [9]. However, these findings differ from the outcomes of previous systematic reviews [5, 6], potentially because studies included in the reviews generally examined younger children (under the age of 3 years), used tools that combined language and cognition, or were from the pre-ART era. Similarly, there was no evidence of an association between HIV exposure and memory at 3.5 years in this cohort. There is scarce research on

early memory development in CHEU, and this domain is often assessed as part of a composite score, and so further studies at older ages are required, particularly given higher order cognitive functions are still developing. Recent studies indicate memory and academic performance may be affected at older ages [15, 32], and so follow up is important.

Interestingly, there was evidence to suggest that male CHEU performed significantly worse than male CHUU. Prior research in this cohort has shown that boys perform worse than girls in early developmental assessments [14]. Similarly, the effects of sex have also been noted in other recent studies, suggesting male CHEU may be at most risk [15, 33]. Research set in the east Asia-Pacific region investigating sex disparities in child development in low- and middle-income country settings suggest that girls aged 3–5 years generally outperform boys on tests of development [34]. It is likely that environmental and psychosocial factors play a role in these differences, along with genetic and hormonal differences. Studies also suggests sexual dimorphism in early brain trajectories [35], and that there is significantly greater variance for several key brain structures in males, including white matter and cerebellar cortex, providing a novel perspective on sex differences in brain maturation [36]. Given reported differences in brain structure between CHEU and CHUU [37], further research using neuroimaging may help to better understand the underlying neuroanatomical pathways.

Investigating the determinants of CHEU neurodevelopment requires a holistic approach [29], including biological influences [38, 39], and environmental factors [40]. Several risk factors for poor neurocognitive development are highlighted in the literature and may impact upon the HIV exposure-outcome relationship [14]. The WHO Nurturing Framework [29] identifies five domains that are crucial to child development: health, nutrition, security, responsive caregiving and opportunities for early learning. The framework acknowledges that these domains are often dependent on socioeconomic status and immediate environmental factors, such as parental characteristics, as well as intrinsic factors such as child age and sex and that the accumulation of interacting factors may lead to the observed cognitive outcomes. Maternal education is frequently highlighted as important for child development [27], and we found an association between higher maternal education levels and better memory function in male CHEU, and expressive language in female CHEU. There is also considerable evidence that poor birth outcomes can adversely affect neurocognitive development [41]. In our exploration of other potential factors, we found an association between birthweight and cognitive outcomes in male CHEU, such that higher birthweight was associated with better outcomes. Birthweight has been found to be a protective factor [14], and conversely low birthweight is a known risk factor for morbidity [4], and may represent cumulative *in utero* insults including nutrition and infectious diseases [28, 42]. Our findings suggest low birthweight CHEU infants may be particularly vulnerable; it is likely that the combination of risk factors (low birthweight, male sex, and HIV exposure) may compound the risk of poor cognitive outcomes. Together, this research highlights the need to identify and protect those children with multiple risk factors. In terms of HIV-related variables, we found that better child outcomes were associated with longer duration of maternal ART (initiation pre-pregnancy versus during pregnancy). Conversely, worse outcomes were associated with high maternal viral load in pregnancy. This supports the existing literature where another South African study found maternal HIV viraemia in pregnancy predicted lower expressive language and motor outcomes and increased delay at 1 year [43]. Separately another study found infants born to mothers initiating ART during pregnancy had smaller subcortical volumes, compared to those initiating ART before conception and increasing ART exposure was protective [44]. Overall, these findings emphasise the importance of optimizing maternal HIV care in pregnancy to improve child outcomes.

This study adds value to the field with demographically comparable unexposed control group, providing a reasonable estimate of the counterfactual. This overcomes common methodological limitations that have hampered many prior studies, namely small sample sizes, unmeasured confounders and unbalanced exposure groups in the context-specific literature. The comprehensive data collected by the DCHS on maternal, environmental, and household characteristics allowed this analysis to control for factors with an established impact on neurocognitive outcomes in children. With a large enough sample size, these factors were included in the analysis without introducing data sparsity. This cohort may also be generalisable to other sub-Saharan contexts and uses the KABC, a well-validated tool [24]. These approaches lend greater specificity for measuring the impact of interventions focused on improving deficits in cognition [45], suggesting the results may be used to evaluate future interventions.

This study has some limitations. Although results held on adjusted analyses, other unmeasured potential confounders may have resulted in residual confounding. Further work needs to be carried out to understand the impact of other variables, including household composition, in the association between HIV exposure and neurocognitive development and to replicate the findings in other settings. While infant feeding practices are an important potentially modifiable factor for the neurocognitive growth in children [46], there was a low prevalence of exclusive breastfeeding in this cohort which may have limited the power to investigate an association with neurocognitive outcomes. A complete-case analysis was used to mitigate missing outcome data and potential selection bias was evaluated. It was noted that missing data was similar across exposure groups and the distribution of demographic and other covariate data for the full cohort was similar to the analytic cohort; results held on sensitivity analyses of each outcome measure. The KABC-II is normed using data from a standardised sample from a high-income setting which creates limitations in this setting, and it will be important to compare with other scales with contextually appropriate norms in the future. However, the tool has been validated in the sub-Saharan context [47], and in young African children affected by HIV [24] and we focused on between-group comparisons. There are also other components of neurocognition such as executive function, motor control and social cognition that were not reported here. Finally, we note that the cohort was recruited in 2012–2015. In considering comparisons to current times, new note that our cohort is representative of women living with HIV nationally [48], with similar sociodemographics [49], and overall ART coverage at the time of study enrolment was comparable to many SSA countries in recent times [1]. Further work is needed to assess generalisability, particularly given the relationship between efavirenz and neurocognition [50, 51] and the introduction of dolutegravir-based ART [52].

Conclusions

This analysis builds upon previous research that identified an association between HIV exposure in children and language development in sub-Saharan Africa, showing continued subtle impairment in expressive language at 3–4 years. This is notable giving the rapidly growing and ageing population of CHEU [1], and indicates the need for strategies to detect language problems early and implement interventions to optimise outcomes. The identified vulnerability of CHEU and other biological and demographic risk factors may help target intervention strategies. Further research should be undertaken to assess whether these results can be replicated in other contexts, focusing on understanding the mechanisms behind adverse neurodevelopment outcomes and paying particular attention to potential sex differences.

Supporting information

S1 Fig. Directed acyclic graph displaying plausible pathways for the association between HIV exposure and neurocognitive development.
(PDF)

S1 Table. Outcome measures.
(PDF)

S2 Table. Maternal, child and household characteristics of the full cohort, by exposure.
(PDF)

S3 Table. Neurocognitive outcomes at 3.5 years compared between CHEU and CHUU in the analytic (complete-case) cohort.
(PDF)

S4 Table. Neurocognitive outcomes at 3.5 years compared between CHEU and CHUU among all those completing each assessment.
(PDF)

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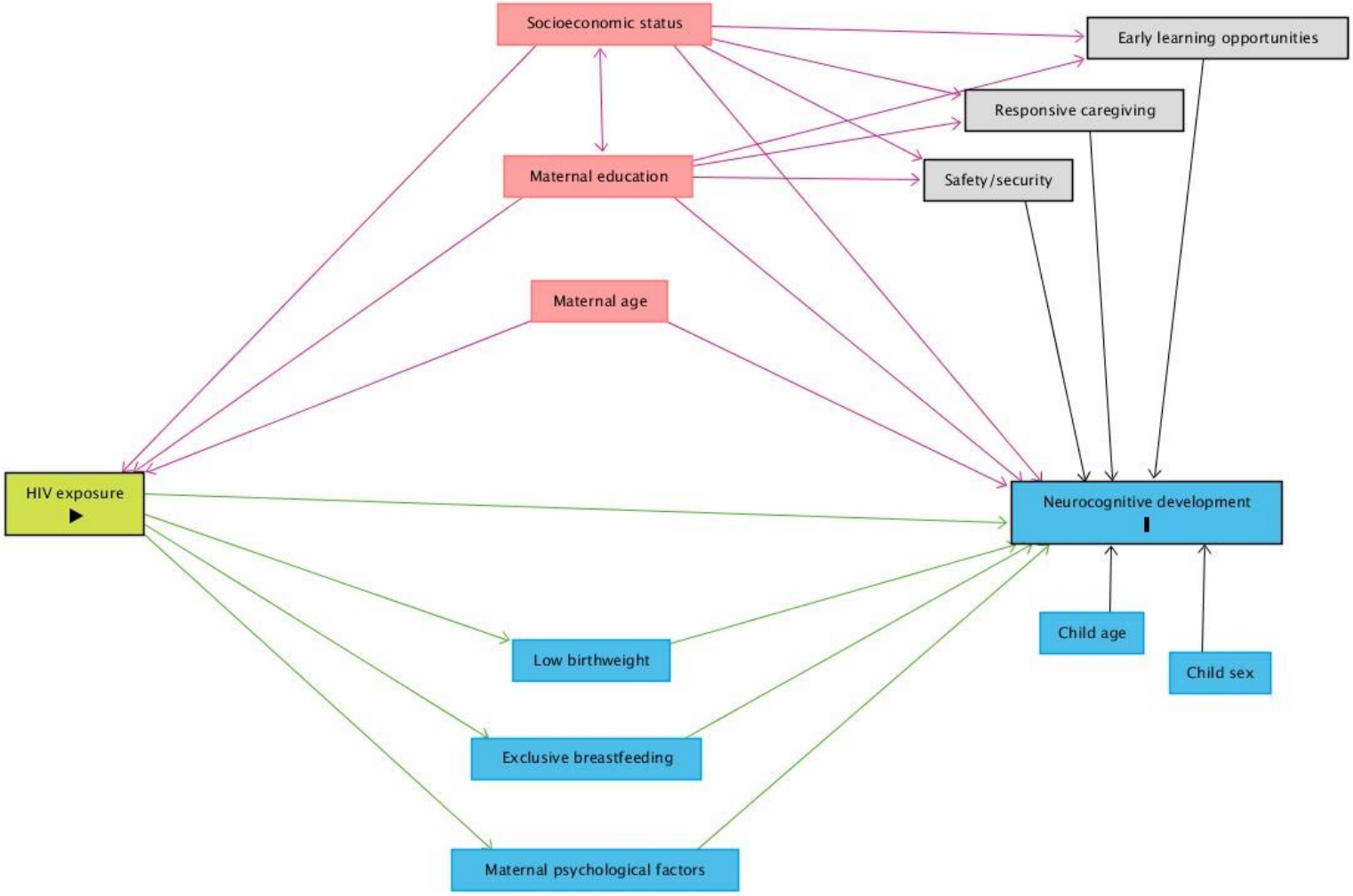
S1 Figure: Directed Acyclic Graph displaying plausible pathways for the association between HIV exposure and neurocognitive development



S1 Table: Outcome measures

Characteristic	Measure	Details
General cognitive function	Kaufman Assessment Battery for Children (KABC-II) - Non-verbal index	The Non-verbal index permits a valid assessment of children who are hearing impaired, have limited English proficiency or have moderate to severe speech or language impairments.
Problem solving	KABC-II Conceptual thinking	The child views a set of 4 or 5 pictures and identifies the one picture that does not belong with the other. Some items present meaningful stimuli, and others use abstract stimuli.
Visual-spatial processing	KABC-II Face recognition	The child attends closely to photographs of one or two faces that are exposed briefly and then selects the correct face or faces, shown in a different pose, from a group photograph.
Visual-spatial problem solving	KABC-II Triangles	For most items, the child assembles several identical foam triangles (blue on one side, yellow on the other) to match a picture of an abstract design; for easier items, the child assembles a set of colourful plastic shapes to match a model constructed by the examiner or shown on an easel.
Working memory/motor sequencing	KABC-II Hand movements	The child copies the examiner's precise sequence of taps on the table with the fist, palm, or side of the hand
Language	KABC-II Expressive Language	The child provides the name of a pictured object presented by the examiner
Memory	KABC-II Atlantis	The examiner teaches the child the nonsense names for fanciful pictures of fish, plants and shells; the child demonstrates learning by pointing to each picture (out of an array of pictures) when it is named.

S2 Table: Maternal, child and household characteristics of the full cohort, by HIV exposure

Variable	Full Cohort (n=1141)	CHEU (n=247)	CHUU (n=894)	P-value
Child sex (female)	553 (48.5)	109 (44.1)	444 (49.7)	0.123
Low Birthweight	176 (15.4)	35 (14.2)	141 (15.8)	0.537
Premature Birth	188 (16.5)	47 (19.0)	141 (15.8)	0.474
Maternal age (yrs)	26.9 (5.68)	29.9 (5.25)	26.1 (5.51)	<0.001
Maternal education				
Primary	85 (7.5)	26 (10.5)	59 (6.6)	<0.001
Some Secondary	609 (53.4)	79 (62.8)	454 (50.8)	
Completed Secondary	374 (32.8)	27 (24.29)	314 (35.1)	
Any Tertiary	73 (6.4)	67 (7.5)	32 (7.5)	
Smoker status				
Non-smoker	261 (22.9)	57 (23.1)	204 (22.8)	0.124
Passive smoker	479 (42.0)	118 (47.8)	361 (40.4)	
Active smoker	351 (30.8)	63 (25.5)	288 (32.2)	
Employed	307 (26.9)	61 (24.7)	246 (27.5)	0.376
Exclusive Breastfeeding for 5 months	167 (14.6)	36 (14.6)	93 (17.7)	0.569
Household income				
<R1000/m	431 (37.8)	96 (38.9)	335 (37.5)	0.411
R1000-R5000/m	556 (48.7)	124 (50.2)	432 (48.2)	
>R5000/m	154 (13.5)	27 (10.9)	127 (14.2)	

Footnote. N(%) for categorical variables, mean (SD) for continuous variables. P-values generated using, chi-squared test for categorical variables and t-test for continuous variables. Percentages calculated out of all data. Missing data: prematurity n=5, breastfeeding n=77, alcohol use n=146, EPDS n=50, smoking n=50, water n=5, toilet n=2. Abbreviations: CHEU: Children who are HIV-exposed uninfected, CHUU: Children who are HIV-unexposed uninfected

S3 Table: Neurocognitive outcomes at 3.5 years compared between CHEU and CHUU in the analytic (complete-case) cohort

Domain	Scores Mean (SD)	Unadjusted mean difference (95% CI)	p-value	Effect size	Adjusted† mean difference (95% CI)	p-value	Effect size
Cognitive function (score)							
CHUU	76.55 (14.77)	Reference	0.637	-	Reference	0.672	-
CHEU	75.77 (13.85)	-0.78 (-4.02, 2.47)		-0.05 (-0.28, 0.17)	-0.72 (-4.06, 2.62)		-0.04 (-0.27, 0.17)
Expressive language							
CHUU	7.23 (2.03)	Reference	0.026*	-	Reference	0.049*	-
CHEU	6.73 (1.79)	-0.50 (-0.94, -0.06)		-0.25 (-0.47, -0.03)	-0.46 (-0.91, -0.02)		-0.23 (-0.45, -0.01)
Memory							
CHUU	7.83 (1.90)	Reference	0.257	-	Reference	0.184	-
CHEU	8.06 (1.45)	0.23 (-0.17, 0.64)		0.13 (-0.09, 0.35)	0.29 (-0.14, 0.71)		0.16 (-0.06, 0.38)
Domain	**Sub-optimal development**	**Unadjusted odds**	**p-value**		**Adjusted† odds ratio**	**p-value**	
Cognitive function							
CHUU	254 (63.5)	1	0.518	-	1	0.474	-
CHEU	65 (67.0)	1.16 (0.73, 1.87)		-	1.20 (0.73, 1.99)		-

Footnote: †Adjusting for child sex, maternal education, maternal age and household income. Effect size measured using Cohen's d. *p<0.05.
Abbreviation: CHUU: Children who are HIV-unexposed uninfected; CHEU: Children who are HIV-exposed uninfected.

S4 Table: Neurocognitive outcomes at 3.5 years compared between CHEU and CHUU among all those completing each assessment

Domain	Scores Mean (SD)	Unadjusted mean difference (95% CI)	p-value	Effect size	Adjusted† mean difference (95% CI)	p-value	Effect size
Cognitive function (score)							
CHUU	77.00 (14.84)	Reference	0.687	-	Reference	0.853	-
CHEU	76.41 (14.52)	-0.59 (-3.50, 2.31)		-0.04 (-0.24, 0.16)	-0.28 (-3.25, 2.69)		-0.02 (-0.22, 0.18)
Expressive language							
CHUU	7.22 (2.02)	Reference	0.012*	-	Reference	0.038*	-
CHEU	6.69 (1.82)	-0.53 (-0.95, -0.12)		-0.27 (-0.48, -0.06)	-0.45 (-0.88, -0.02)		-0.23 (-0.44, -0.02)
Memory							
CHUU	7.89 (1.89)	Reference	0.444	-	Reference	0.261	-
CHEU	8.02 (1.45)	0.13 (-0.20, 0.46)		0.07 (-0.11, 0.26)	0.20 (-0.15, 0.55)		0.11 (-0.07, 0.29)
Domain	**Sub-optimal development**	**Unadjusted odds**	**p-value**		**Adjusted† odds ratio**	**p-value**	
Cognitive function							
CHUU	331 (62.57)	1	0.732	-	1	0.756	-
CHEU	79 (64.23)	1.07 (0.71, 1.62)		-	1.07 (0.69, 1.65)		-

Footnote: †Adjusting for child sex, maternal education, maternal age and household income. Effect size measured using Cohen's d. *p<0.05.
Abbreviation: CHUU: Children who are HIV-unexposed uninfected; CHEU: Children who are HIV-exposed uninfected

Appendix XII: A neurometabolic pattern of elevated myo-inositol in children who are HIV-exposed and uninfected: a South African birth cohort study *(co-first author research paper)*

Bertran-Cobo C^, Wedderburn CJ^, Robertson FC, Subramoney S, Narr KL, Joshi SH, Roos A, Rehman AM, Hoffman N, Zar HJ, Stein DJ, Donald KA. A neurometabolic pattern of elevated myo-inositol in children who are HIV-exposed and uninfected: a South African birth cohort study. Front Immunol 2022;13:800273 *(^shared first authorship)*

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A Neurometabolic Pattern of Elevated Myo-Inositol in Children Who Are HIV-Exposed and Uninfected: A South African Birth Cohort Study

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Introduction: Exposure to maternal HIV in pregnancy may be a risk factor for impaired child neurodevelopment during the first years of life. Altered neurometabolites have been associated with HIV exposure in older children and may help explain the mechanisms underlying this risk. For the first time, we explored neurometabolic profiles of children who are HIV-exposed and uninfected (CHEU) compared to children who are HIV-unexposed (CHU) at 2-3 years of age.

Methods: The South African Drakenstein Child Health Study enrolled women during pregnancy and is following mother-child pairs through childhood. MRI scans were acquired on a sub-group of children at 2-3 years. We used single voxel magnetic resonance spectroscopy to measure brain metabolite ratios to total creatine in the parietal grey matter, and left and right parietal white matter of 83 children (36 CHEU; 47 CHU). Using factor analysis, we explored brain metabolite patterns in predefined parietal voxels in these groups using logistic regression models. Differences in relative concentrations of individual metabolites (n-acetyl-aspartate, myo-inositol, total choline, and glutamate) to total creatine between CHEU and CHU groups were also examined.

Results: Factor analysis revealed four different metabolite patterns, each one characterized by covarying ratios of a single metabolite in parietal grey and white matter. The cross-regional pattern dominated by myo-inositol, a marker for glial

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reactivity and inflammation, was associated with HIV exposure status (OR 1.63; 95% CI 1.11–2.50) which held after adjusting for child age, sex, and maternal alcohol use during pregnancy (OR 1.59; 95% CI 1.07 –2.47). Additionally, higher relative concentrations of myo-inositol to total creatine were found in left and right parietal white matter of CHEU compared to CHU (p=0.025 and p=0.001 respectively).

Discussion: Increased ratios of myo-inositol to total creatine in parietal brain regions at age 2-3 years in CHEU are suggestive of early and ongoing neuroinflammatory processes. Altered relative concentrations of neurometabolites were found predominantly in the white matter, which is sensitive to neuroinflammation, and may contribute to developmental risk in this population. Future work on the trajectory of myo-inositol over time in CHEU, alongside markers of neurocognitive development, and the potential for specific neurodevelopmental interventions will be useful.

Keywords: HIV exposure, magnetic resonance spectroscopy, neuroinflammation, brain development, myo-inositol

INTRODUCTION

Human immunodeficiency virus (HIV) infection remains a major public health concern worldwide, with 37.7 million people reported to be living with HIV globally (1). Of these, an estimated 25.3 million people live in sub-Saharan Africa. The widespread roll-out of antiretroviral therapy (ART) and expansion of ART programmes for prevention of mother-to-child transmission (PMTCT) have led to dramatic declines in vertical transmission rates to less than 5% during recent years (2). Globally, the estimated number of new infections in children aged 0 to 14 years has decreased by more than 60% since the year 2000 (3). However, progress in the eradication of paediatric HIV infection has revealed a concern that children who are HIV-exposed and uninfected (CHEU) remain a vulnerable population (2, 4). Approximately 15.4 million children worldwide are CHEU, 13.8 million of whom live in sub-Saharan Africa (1), with the highest number of CHEU residing in South Africa (3). Due to expanding accessibility of both ART and PMTCT programmes this population is increasing in number, however, the implications of HIV and ART exposure as risk factors for long-term child health and development are less well defined (4, 5).

Meta-analyses have found that CHEU are at a greater risk of all-cause mortality and worse developmental outcomes within the early years of life, compared to children who are HIV-unexposed (CHU) (6, 7). In sub-Saharan Africa, recent studies have described HIV exposure to be associated with neurodevelopmental delay (8–11) in children younger than 3 years of age. However, there is inconsistency across studies and settings, and others have reported CHEU having similar outcomes to CHU (12, 13).

There are a number of hypothesised mechanisms by which HIV exposure may impact paediatric brain development. As argued in the two-hit model of early brain damage, inflammatory intrauterine conditions may increase vulnerability of the developing brain to postnatal adverse events (14, 15). Since chronic inflammation can persist in HIV infection despite ART, women living with HIV may have immune dysregulation during pregnancy (16, 17). This may prime the developing brain to trigger exaggerated inflammatory responses against future insults, compromising typical neurobiological development (18–20). Immunological studies suggest the immune system of CHEU is altered compared to that of CHU (17, 21), some revealing proinflammatory immune profiles from birth to 2 years of age (22, 23). Neurobiological development in CHEU may therefore be affected by maternal immune dysregulation during pregnancy, however, studies of early neurometabolic development are lacking.

Exposure to ART has also been associated with potential neurotoxicity (24). Although maternal ART and child prophylaxis are important to prevent HIV transmission, potential metabolic and neurological consequences have been reported (25). Furthermore, environmental stressors are known to influence long term neurodevelopmental outcomes during the period from conception to 2 years of age, and psychosocial risk factors such as maternal antenatal depression and alcohol use in pregnancy may play a key role in child development (26, 27). Overall, there remains a gap in understanding the neurobiological consequences of HIV exposure in the context of high-risk environments.

Neuroimaging studies provide a key opportunity to examine HIV exposure-related neuropathophysiology (28), with reports describing white matter and grey matter differences between newborns who are HEU compared to HU (29, 30) and white matter abnormalities in older children who are HEU (31). Amongst the existing techniques, magnetic resonance spectroscopy (MRS) is a powerful approach, since it provides *in vivo* measurements of neurometabolites in specified brain regions. MRS profiles of the neurotypical brain during childhood are well characterized (32, 33), and this technique has previously been used to describe metabolite alterations in children older than 2 years with perinatal infection or exposure to HIV (34–36). Only one cohort study to date has examined neurometabolic characteristics of CHEU, reporting metabolite alterations in the basal ganglia at age 9 years, and in the frontal grey matter (GM)

and peritrigonal white matter (WM) at age 11 years, compared to CHU (35, 36). MRS data are suitable for dimensionality reduction methods like factor analysis, which groups similar variables into a smaller number of dimensions. Through the combination of metabolite measurements across different brain regions, this method identifies metabolic patterns that underlie latent neurobiological processes. Factor analysis has previously been used in MRS studies to identify metabolic patterns within the context of HIV-related illness (36–38).

The aim of our study was to explore differences in brain metabolites in a well-characterized cohort of CHEU and CHU from similar sociodemographic conditions at 2-3 years of age, using MRS and factor analysis. We hypothesised that CHEU would have altered neurometabolic profiles compared to CHU in GM and WM, related to factors associated with inflammation.

METHODS

Participants

The Drakenstein Child Health Study (DCHS) is a population-based birth cohort study in a peri-urban area of the Western Cape, South Africa, focused on investigating the early-life determinants of child health, development and illness (39–41). The local population is a low socioeconomic community with a high prevalence of several health risk factors including HIV infection.

The DCHS enrolled pregnant women between 2012 and 2015 during their second trimester of gestation and currently follows the mother-child pairs into middle childhood. Inclusion criteria for enrolment were a minimum age of 18 years, gestational period of 20–28 weeks, planned attendance at one of the two clinics and intention to remain in the area. All mothers gave written informed consent.

A subset of children enrolled in the DCHS participated in a longitudinal neuroimaging sub-study. As part of the neuroimaging sub-study, children who had undergone neonatal imaging (41) were invited to be scanned at 2-3 years. In addition, children not imaged at birth were also included selecting for risk factors (maternal HIV and alcohol use during pregnancy) to ensure a representative sample of a high-risk population, along with a randomly selected comparison group. These children were currently active in the study and living in the area. Exclusion criteria applied to children for this sub-study were: medical comorbidities such as congenital abnormality, genetic syndrome, or neurological disorder; low Apgar score (<7 at 5 minutes); neonatal intensive care admission; history of maternal use of illicit drugs during pregnancy; child HIV infection; and MRI contra-indications including cochlear implants (42).

Sociodemographic Data Collection

The HIV status of enrolled mothers was confirmed *via* routine testing during pregnancy and re-checked every 12 weeks, in accordance with the Western Cape PMTCT guidelines (43). Children who were HIV-exposed were tested at age 6 weeks, 9 months, and 18 months using PCR, rapid antibody, or ELISA tests as per guidance. CHEU were confirmed to be negative for HIV at the age of 18 months, or once the mother had stopped breastfeeding if this lasted more than 18 months. CHU were defined as children born to mothers without HIV infection. Mothers living with HIV received ART according to PMTCT guidelines at the time. CHEU were prescribed post-exposure prophylaxis from birth (44). Maternal CD4 cell count and viral load data during pregnancy were abstracted from clinical records and the online National Health Laboratory Service system, collected as part of clinical care protocols. The lowest maternal CD4 cell count within 1 year before child's birth and 3 months after birth was used to maximise numbers.

Sociodemographic and maternal psychosocial data were collected between weeks 28 and 32 of gestation, through interviews and questionnaires adapted from the South African Stress and Health study (39, 40). Infant birthweight and markers of poor nutrition were also collected, in accordance with the World Health Organization (WHO) Z-score guidelines (45). Stunting was defined as low child height-for-age, underweight as low child weight-for-age, and wasting as low child weight-for-length, all calculated as Z-scores lower than -2 of the WHO Child Growth Standards median. Maternal alcohol use during pregnancy was assessed using the Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST), and data on moderate-severe alcohol use in pregnancy was retrospectively collected, forming a dichotomous measure (41). Maternal smoking during pregnancy was determined through self-reporting. Maternal depression was assessed with the Edinburgh Postnatal Depression Scale.

Magnetic Resonance Spectroscopy Protocol

Participants in the neuroimaging sub-study underwent a multimodal magnetic resonance imaging (MRI) protocol without sedation, performed between January 2016 and September 2018 at Groote Schuur Hospital, University of Cape Town, on a 3 Tesla Siemens Skyra 70cm diameter bore whole body MRI scanner (Erlangen, Germany) using a 32-channel head coil (42). Once informed consent was acquired from the mother and the child had fallen into deep sleep, children were carried into the scanner, positioned carefully with pillows, blankets, and ear protection. MRS data acquisition was performed during natural sleep, and a trained study staff member remained in the scanner room during the entire session in case the child woke (42).

The MRS protocol was performed by well-trained radiographers who were blinded to the children's HIV exposure status. It consisted of a high-resolution T1-weighted multi-echo magnetisation prepared rapid gradient echo acquisition (MEMPRAGE (46); sagittal orientation, repetition time (TR) 2530 ms, echo times (TE) = 1.69/3.54/5.39/7.24 ms, flip angle 7.0°, voxel size 1.0 x 1.0 x 1.0 mm^3, inversion time (TI) 1100 ms, field of view (FOV) 224 x 224 x 176 mm, 176 slices, scan time 5 min 21 s) and single voxel Point RESolved Spectroscopy (PRESS; TR 2000 ms, TE 30 ms, 128 averages, voxel size 25 x 25 x 25 mm^3, vector size 1024, spectral bandwidth

1200 Hz, scan time 6 min) with Chemical Shift Selective (CHESS) water suppression. A water reference was acquired without using CHESS. Shimming was automatically performed over the voxel volume (with use of the scanner's advanced adjustments) and manually adjusted if necessary, to reduce the spectral linewidths reported by the scanner. Voxel 1 was targeted at the midline parietal GM, voxels 2 and 3 were targeted at left and right parietal WM respectively (**Figure 1**).

Magnetic Resonance Spectroscopy Data Processing

MRS voxels were registered to the T1-weighted structural image with use of MATLAB software (MATLAB. Natick, Massachusetts: The MathWorks Inc.; 2017). Segmentation of the structural image into GM, WM, and cerebrospinal fluid (CSF) was performed using Statistical Parametric Mapping (SPM12) software (www.fil.ion.ucl.ac.uk/spm) to determine tissue composition for each voxel.

LCModel software (version 6.3-1) (47) was run to fit the raw spectral data for quantification, using the appropriate water reference for eddy current correction. Relative concentrations (ratios) to the reference signal, creatine and phosphocreatine (Cr+PCr), were determined for n-acetyl-aspartate (NAA/Cr+PCr), myo-inositol (Ins/Cr+PCr), total choline (glycerophosphocholine and phosphocholine, GPC+PCh/Cr+PCr), and glutamate (Glu/Cr+PCr). Quality of spectra was inspected visually and assessed in terms of full width at half maximum (FWHM) and signal-to-noise ratio (SNR), and Cramér-Rao lower bounds (CRLB) given by LCModel. Spectra with FWHM values greater than 0.08, and SNR values lower than 10 were considered of low quality and therefore excluded.

The four metabolites considered in our study have been characterized in terms of clinical significance in prior studies, from birth through childhood (32, 33). N-acetyl-aspartate is most commonly considered to be a marker for neuronal health or density in the developing brain (32, 33). While we note that

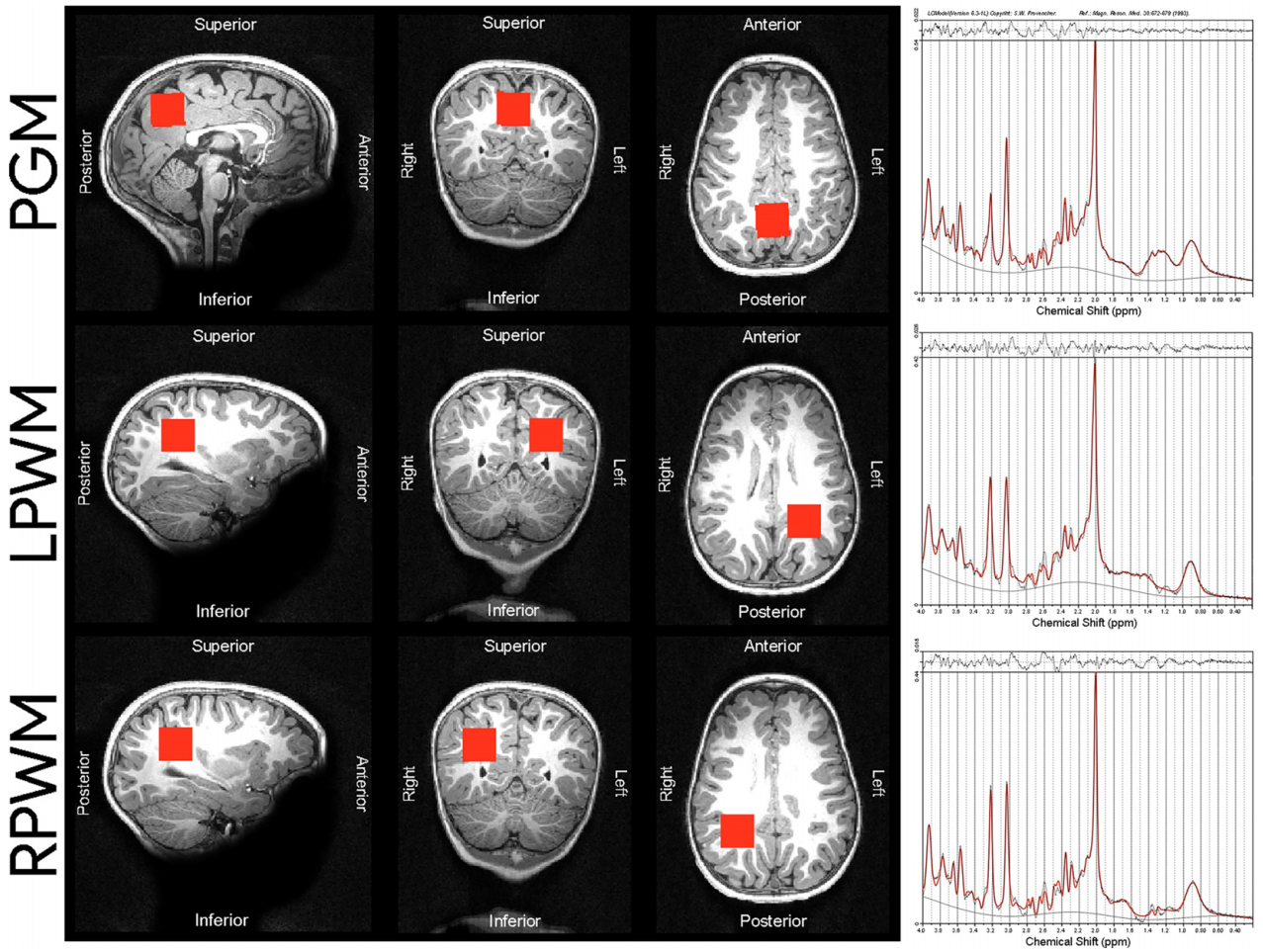

FIGURE 1 | Voxel placements and sample spectra of median SNR and LCModel output from the 3T scanner used in our cohort of children. The following median SNR were obtained for each voxel placement: PGM median SNR = 34, LPWM median SNR = 35, RPWM median SNR = 33. PGM, parietal grey matter; LPWM, left parietal white matter; RPWM, right parietal white matter; SNR, signal-to-noise ratio; ppm, parts per million.

the role of n-acetyl-aspartate in mature brain remains to be fully established and recognise that n-acetyl-aspartate may also play additional roles, such as contributing to myelin synthesis in the mature brain (48), the evidence for this is currently limited. Myo-inositol is considered a marker for glial reactivity, gliosis and neuroinflammation. Total choline is associated with myelination, membrane synthesis and membrane maturation in the WM. Glutamate, the main excitatory neurotransmitter in the brain, is considered a marker for neuronal function involved in many neurobiological and behavioural processes during brain development (32, 33).

Statistical Analysis

Sociodemographic characteristics of the mother-child pairs were reported as mean (± SD) for continuous data, or absolute frequencies (%) for categorical data. Continuous data was assessed for normality using Shapiro-Wilk tests. Comparisons between CHEU and CHU were made using *t*-tests or Wilcoxon tests for normally and non-normally distributed continuous data, respectively, and X^2 tests for categorical data.

Factorability of MRS data was assessed using Bartlett sphericity and Kaiser-Meyer-Olkin (KMO) tests. Factor analysis was carried out with use of a maximum likelihood approach and varimax rotation, and Root Mean Square Errors of Approximation (RMSEA) of less than 0.05 were considered to indicate statistical goodness of fit of the model. As proposed by Yiannoutsos and colleagues (38), factor scores were constructed for MRS data using a weighted linear combination of all 12 variables (the ratios of 4 metabolites to total creatine in each of the 3 voxels), multiplying each metabolite concentration by its associated factor loading and summing all products to form each of four factor scores (38).

To determine whether the brain metabolic patterns could predict HIV exposure, the factor scores obtained from brain metabolite ratios were included as independent variables in logistic regression models, to estimate odds ratios (OR) and 95% confidence intervals (CI). Both unadjusted and multivariable models were created. Potential confounders were chosen *a priori* due to their reported influence in neurometabolic or neurobehavioral outcomes in children. These included child age (32, 33), child sex (27, 49), and maternal alcohol use during pregnancy (50, 51).

Sensitivity analyses were performed to examine the effect of sociodemographic characteristics that showed significant differences ($p<0.05$) between CHEU and CHU, by additionally adjusting for these variables: maternal age of delivery, and maternal depression during pregnancy. Despite having similar values between groups, infant birthweight was also included in the sensitivity analysis, since its role as confounder or mediator in the causal pathway of maternal HIV infection and child developmental outcomes may vary across settings (52).

Region-specific analyses were run for each metabolite ratio, to explore differences between CHEU and CHU. Comparisons between groups were made using unadjusted and adjusted linear regression analyses with robust standard errors. Child age, child sex, and maternal alcohol use during pregnancy were included as covariates. To account for the presence of GM in voxels targeted at parietal WM, GM percentage was included as a confounder in sensitivity analyses.

Lastly, we planned to examine the association of each child metabolite pattern identified from factor analysis, with maternal immune status during pregnancy and time of maternal ART initiation, using multinomial logistic regression to estimate relative risk ratios. For maternal immune status during pregnancy, a categorical variable was created with the following levels: lowest maternal CD4 cell count during pregnancy ≤500 cells/mm^3 versus >500 cells/mm^3 in CHEU. Similarly, for maternal ART initiation, a categorical variable was created examining maternal ART initiation before pregnancy versus during pregnancy. CHU was used as the reference in both models. A Cramér's V test was run to check for multicollinearity between the categorical variables.

Statistical analyses were performed in R with RStudio software (version 1.2.5033) (53). P values of less than 0.05 (two-tailed) were considered statistically significant.

RESULTS

Cohort and Demographic Characteristics

A total of 1143 mother-child pairs were enrolled in the DCHS. A subset of 156 children had MRS imaging at age 2-3 years. Of these, 143 had a successful MRS acquisition from the parietal grey matter voxel (first voxel in the data acquisition protocol), 134 from the left parietal WM voxel (acquired second), and 92 from the right parietal WM voxel (acquired third and last). A total of 9 participants were excluded from the study after inspection of obtained MRS data due to low quality of spectra in at least one of the three voxels. Our final complete-case cohort included 83 children (36 CHEU, 47 CHU) who had usable metabolite data for all three voxels (i.e., GM, left and right WM) and complete covariate data (**Figure 2**).

Socioeconomic characteristics of the complete-case cohort of children were comparable between groups. Mothers living with and without HIV had similar household incomes, education, employment status, marital status, hospitalization rates and smoking or alcohol use during pregnancy (**Table 1**). However, mothers living with HIV were older at delivery and, among those with available data (N=28 CHEU, N=42 CHU), there were lower rates of depression compared to their uninfected counterparts. Weight at birth was similar for CHEU and CHU. Exclusive breastfeeding duration was comparable between groups, as was the proportion of children with WHO markers for poor nutrition. All mothers living with HIV received first-line three-drug ART regimens, whereas post-exposure prophylaxis for CHEU included nevirapine (77.7%) or nevirapine and zidovudine (22.3%). The complete-case cohort and the original subset of 156 children were similar in terms of sociodemographic characteristics (**Supplementary Table 1**).

Metabolite Patterns of CHEU and CHU

Fractional tissue composition in each of the three voxels of the complete-case cohort did not differ between groups. The percentage of GM in the voxel targeted at parietal GM was

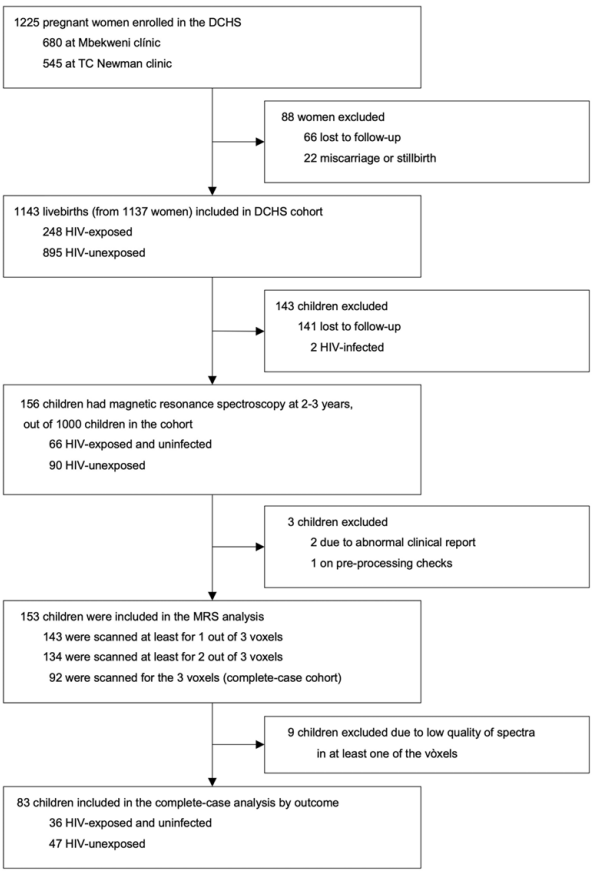

FIGURE 2 | DCHS flowchart for MRS sub-study. Exclusion criteria for the MRS nested sub-study are described in the methodology section.

≈77% for both CHEU and CHU, while the voxels targeted at left and right parietal WM contained ≈52% of WM in both groups (**Table 2**). For all spectral fits the CRLB for NAA/Cr+PCr were ≤7%, for Ins/Cr+PCr ≤6%, for GPC+PCh/Cr+PCr ≤6%, and for Glu/Cr+PCr ≤8%.

Bartlett sphericity and KMO tests confirmed the factorability of our data. Subsequent factor analysis identified four factors (RMSEA < 0.05), which accounted for 69% of data variability (**Table 3**). Each factor is a metabolic pattern composed of loadings associated with each of the metabolite ratios (/Cr+PCr), where a large loading (>0.6) indicates a strong contribution of a certain metabolite ratio to the factor. Factor 1 was composed of large loadings of NAA/Cr+PCr across all three brain regions and a strong contribution of Glu/Cr +PCr in the voxel targeted at parietal GM. Factor 2 was dominated by large loadings of Ins/Cr+PCr across brain regions. Factor 3 was composed of large loadings of GPC+PCh/Cr+PCr in the voxels targeted at left and right parietal WM, and a medium contribution (0.552) of the same metabolite in the voxel targeted at parietal GM. Factor 4 was characterized by large loadings of Glu/Cr+PCr in the voxel targeted at right parietal WM and a medium contribution (0.530) of the same metabolite ratio in the voxel targeted at left parietal WM.

In both unadjusted and adjusted logistic regression models, HIV exposure was significantly predicted by factor 2 (dominated by Ins/Cr+PCr across regions), with an OR estimate of 1.63 (95% CI 1.11 - 2.50) and adjusted OR 1.59 (95% CI 1.07 - 2.47), respectively (**Table 4**). None of the remaining three factors predicted HIV exposure. Sensitivity analyses revealed similar results when separately adjusting for maternal age at delivery, maternal depression during pregnancy and infant birthweight, with HIV exposure being significantly predicted by factor 2 (**Supplementary Table 2**).

Region-Specific Relative Concentrations of Metabolites to Total Creatine in CHEU and CHU

Unadjusted analyses for each individual metabolite relative concentration to total creatine and brain region revealed significantly higher ratios of Ins/Cr+PCr in left ($p = 0.025$) and right parietal WM ($p = 0.001$) of CHEU, compared to their unexposed peers. Levels of Glu/Cr+PCr in the right parietal WM of CHEU were also significantly higher than those of CHU ($p = 0.034$) (**Figure 3** and **Supplementary Table 3**).

The adjusted analyses did not substantially modify the results obtained for Ins/Cr+PCr ($p = 0.004$) and Glu/Cr+PCr ($p = 0.015$) in the right parietal WM, while group differences in Ins/Cr+PCr ($p = 0.066$) in the left parietal WM fell short of our selected threshold for statistical significance. Results remained similar for all metabolite ratios after accounting for the percentage of GM in WM voxels (data not shown).

Association of Maternal Immune Status and ART Initiation With Child Metabolite Patterns

Maternal immune status and ART initiation variables were found to be co-linear in this sub-group (correlation coefficient >0.7, Cramér's V test). Further, given only 72% mothers of CHEU children in this sample had CD4 cell counts taken during pregnancy, we were unable to run multinomial logistic regression using these variables as due to small sample size and missing data we recognized that our ability to draw valid conclusions from this analysis would be limited.

DISCUSSION

Our study is the first to describe the impact of HIV exposure without infection on brain metabolites at 2-3 years of age in a well-characterised cohort of children living in a LMIC setting. By combining MRS data from parietal grey and white matter regions using a factor analysis approach, we demonstrate a neurometabolite pattern of elevated Ins/Cr+PCr in the parietal brain regions of CHEU; this elevation is suggestive of neuroinflammatory processes.

Factor analysis identified four metabolic patterns in the parietal brain regions of our young cohort. Although all factors represent a weighted combination of all metabolite ratios to total creatine in each region, each factor was characterized by large

TABLE 1 | Sociodemographic characteristics of children included in the MRS complete-case analysis, according to HIV exposure.

	CHEU (N = 36)	CHU (N = 47)	p value
	Mean (±SD) or n/N (%)	Mean (SD) or n/N (%)	
Child age at scan (in months)	33.78 (±1.83)	34.15 (±1.75)	0.35
Sex			0.14
Male	25/36 (69.44%)	24/47 (51.06%)	
Female	11/36 (30.55%)	23/47 (48.93%)	
Monthly household income (in ZAR)			0.49
< 1000	12/36 (33.33%)	17/47 (37.17%)	
1000 - 5000	23/36 (63.88%)	26/47 (55.31%)	
> 5000	1/36 (2.77%)	4/47 (8.51%)	
Maternal education			0.82
Primary	3/36 (8.33%)	3/47 (6.38%)	
Some secondary	22/36 (61.11%)	26/47 (55.31%)	
Completed secondary	10/36 (27.77%)	15/47 (31.91%)	
Tertiary	1/36 (2.77%)	3/47 (6.38%)	
Employed mother	9/36 (25%)	9/47 (19.14%)	0.70
Maternal relationship status (partnered)	19/35 (54.28%)	17/47 (36.17%)	0.22
Maternal age at delivery (in years)	29.89 (±4.37)	25.65 (±5.06)	0.0001*
Gestational age at delivery (in weeks)	38.61 (±2.27)	38.85 (±2.86)	0.67
Premature birth (< 37 weeks' gestation)	5/36 (13.88%)	6/47 (12.76%)	1.00
Birthweight (in g)	3030 (±501.76)	3132 (±622.48)	0.40
Nutritional status at 2 years old			
Stunting (height-for-age Z-score < -2)	5/31 (16.13%)	5/41 (12.19%)	0.89
Underweight (weight-for-age Z-score < -2)	2/31 (6.45%)	1/41 (2.44%)	0.80
Wasting (weight-for-length Z-score < -2)	0/31 (0%)	0/41 (0%)	–
Maternal hospitalization during pregnancy	3/36 (8.33%)	4/47 (8.51%)	1.00
Maternal smoking during pregnancy	7/36 (19.44)	11/46 (23.91)	0.67
Maternal alcohol use during pregnancy	3/35 (8.57%)	10/46 (21.74%)	0.20
Maternal depression during pregnancy	1/28 (3.57%)	11/42 (26.19%)	0.032*
Exclusive breastfeeding duration (in months)	1.919 (±2.25)	2.180 (±1.47)	0.54
Maternal HIV diagnosis timepoint			
Before pregnancy	26/36 (72.22%)		
During pregnancy	10/36 (27.77%)		
Maternal lowest CD4 cell count[§] during pregnancy			
≤ 500 cells/mm^3	12/26 (46.15%)		
> 500 cells/mm^3	14/26 (53.85%)		
Highest maternal viral load during pregnancy			
(undetectable) < 40 copies/ml	25/29 (86.20%)		
40 - 1000 copies/ml	2/29 (6.90%)		
>1000 copies/ml	2/29 (6.90%)		
Antiretroviral therapy initiation			
Before pregnancy	20/36 (55.55%)		
During pregnancy	16/36 (44.44%)		
First-line antiretroviral therapy during pregnancy			
Fixed dose combination (Efavirenz+ Emtricitabine + Tenofovir)	33/36 (91.66%)		
Lamivudine + Zidovudine + Nevirapine	2/36 (5.55%)		
Lamivudine + Zidovudine + Efavirenz	1/36 (2.77%)		
Infant prophylaxis			
Nevirapine alone	28/36 (77.77%)		
Nevirapine and zidovudine	8/36 (22.22%)		

*Data are mean (±SD) or n/N (%). *p<0.05. Percentages calculated out of available data. Continuous data was assessed for normality using Shapiro-Wilk tests. Comparisons between CHEU and CHU were made using t-tests or Wilcoxon tests for normally and non-normally distributed continuous data, respectively, and X^2 tests with Yates correction for categorical data. Missing data: maternal relationship status (N = 1 in the CHEU group); nutritional conditions at 2 years old (N = 5 in the CHEU group, N = 6 in the CHU group); maternal smoking during pregnancy (N = 1 in the CHU group); maternal alcohol use during pregnancy (N = 1 in the CHEU group, N = 1 in the CHU group); maternal depression during pregnancy (N = 8 in the CHEU group, 5 in the CHU group); maternal CD4 cell count in pregnancy (N = 10); highest maternal viral load during pregnancy (N = 7). [§]The lowest maternal CD4 cell count within 1 year before birth and 3 months after birth was used to maximise numbers. CHEU, children who are HIV-exposed and uninfected; CHU, children who are HIV-unexposed; ZAR, South African Rand; WHO, World Health Organization.*

contributions from a certain metabolite ratio grouped across brain regions with generally small contributions from the other metabolite ratios. Based on prior studies of paediatric MRS (32, 33), we proposed the following interpretations: Factor 1 was interpreted as a metabolic pattern for neuronal health or integrity, due to high loadings of NAA/Cr+PCr across brain regions. It also contained a strong contribution from Glu/Cr +PCr in parietal grey matter, suggesting that glutamate may

TABLE 2 | Fractional tissue composition in each defined MRS voxel, according to HIV exposure.

Voxel	CHEU (N = 36)			CHU (N = 47)		
	% Grey matter	% White Matter	% CSF	% Grey matter	% White Matter	% CSF
Parietal grey matter	**77.9** (±4.2)	12.9 (±2.8)	9.2 (±3.2)	**77.2** (±4.5)	14.1 (±2.8)	8.7 (±2.9)
Left parietal white matter	45.2 (±8.8)	**52.1** (±8.9)	2.7 (±1.6)	46.8 (±7.0)	**51.1** (±7.5)	2.1 (±1.2)
Right parietal white matter	46.2 (±8.7)	**51.9** (±9.2)	1.9 (±1.3)	46.1 (±6.6)	**52.5** (±7.0)	1.4 (±0.8)

Data is displayed as mean (±SD) percentages. Bold percentages indicate targeted tissue in each voxel. Data was assessed for normality using Shapiro-Wilk tests. Comparisons between CHEU and CHU were made using t-tests or Wilcoxon tests for normally and non-normally distributed data, respectively. All p values were greater than 0.05 (data not shown). CHEU, children who are HIV-exposed and uninfected; CHU, children who are HIV-unexposed; CSF, cerebrospinal fluid.

TABLE 3 | Factor loadings.

Voxel	Metabolite	Factor Loading			
		Factor 1	Factor 2	Factor 3	Factor 4
PGM	**Glu/Cr+PCr**	**0.745**	-0.044	0.036	0.314
	Ins/Cr+PCr	-0.111	**0.767**	0.062	-0.208
	NAA/Cr+PCr	**0.911**	-0.145	-0.052	0.025
	GPC+PCh/Cr+PCr	-0.264	0.211	**0.552**	0.007
LPWM	**Glu/Cr+PCr**	0.439	-0.034	0.100	**0.530**
	Ins/Cr+PCr	-0.182	**0.906**	0.015	0.003
	NAA/Cr+PCr	**0.889**	-0.116	0.053	0.159
	GPC+PCh/Cr+PCr	0.113	-0.086	**0.821**	0.131
RPWM	**Glu/Cr+PCr**	0.151	0.008	-0.043	**0.883**
	Ins/Cr+PCr	-0.111	**0.823**	0.001	0.168
	NAA/Cr+PCr	**0.692**	-0.208	-0.029	0.072
	GPC+PCh/Cr+PCr	0.104	-0.005	**0.862**	-0.115

Bartlett sphericity and Kaiser-Meyer-Olkin tests were performed and confirmed that a factor analysis approach was suitable for our data. Factor analysis identified four main metabolic patterns (RMSEA < 0.05), which accounted for 69% of data variability and are displayed in this table. Factor loadings in bold represent the main components of each metabolic pattern. PGM, parietal grey matter; LPWM, left parietal grey matter; RPWM, right parietal white matter; NAA, n-acetyl-aspartate; Ins, myo-inositol; GPC+PCh, total choline (glycerophosphocholine + phosphocholine); Glu, glutamate;/Cr+PCr, relative to creatine + phosphocreatine.

TABLE 4 | Logistic regression analysis of factor scores as predictors for HIV exposure.

	Mean factor score		Unadjusted logistic regression			Adjusted logistic regression*		
	CHEU(N = 36)	CHU(N = 47)	OR	Confidence interval (95%)	P value	OR	Confidence interval (95%)	P value
Factor 1 (NAA)	-0.182	0.139	0.72	0.45 – 1.12	0.14	0.72	0.44 – 1.50	0.18
Factor 2 (Ins)	**0.368**	**-0.282**	**1.63**	**1.11 – 2.50**	**0.017**	**1.59**	**1.07 – 2.47**	**0.029**
Factor 3 (GPC+PCh)	-0.030	0.023	0.91	0.51 – 1.59	0.80	0.82	0.42 – 1.55	0.54
Factor 4 (Glu)	0.097	-0.074	1.28	0.76 – 2.21	0.35	1.41	0.81 – 2.56	0.23

*Odds ratios (OR) greater than 1 indicate an increased likelihood of association between a certain metabolite pattern and HIV exposure. Bold data represents statistically significant associations. *Adjusted for child age, child sex, and maternal alcohol use during pregnancy.*

NAA, metabolite pattern dominated by n-acetyl-aspartate ratios; Ins, metabolite pattern dominated by myo-inositol ratios; GPC+PCh, metabolite pattern dominated by total choline (glycerophosphocholine + phosphocholine) ratios; Glu, metabolite pattern dominated by glutamate ratios; CHEU, children who are HIV-exposed and uninfected; CHU, children who are HIV-unexposed.

covary with n-acetyl-aspartate in certain regions and therefore with number or density of neurons. Factor 2 (dominated by Ins/Cr+PCr loadings across all regions) was considered an inflammatory pattern for neuroinflammation or gliosis; and Factor 3 (characterized by GPC+PCh/Cr+PCr across brain regions) was interpreted as a pattern for membrane maturation (32, 33). Factor 4 was dominated by Glu/Cr+PCr across WM regions. This made it challenging to assign an interpretation distinct from that of Factor 1. However, given the role of glutamate in neurocognitive processes including memory, sensory and motor processing (see Blüml et al. and references) (33), Factor 4 was broadly interpreted as a pattern for neuronal function.

We found the inflammatory pattern was associated with HIV exposure, both in the unadjusted and adjusted logistic regression models. In the neurotypical brain, levels of the glial marker Ins/

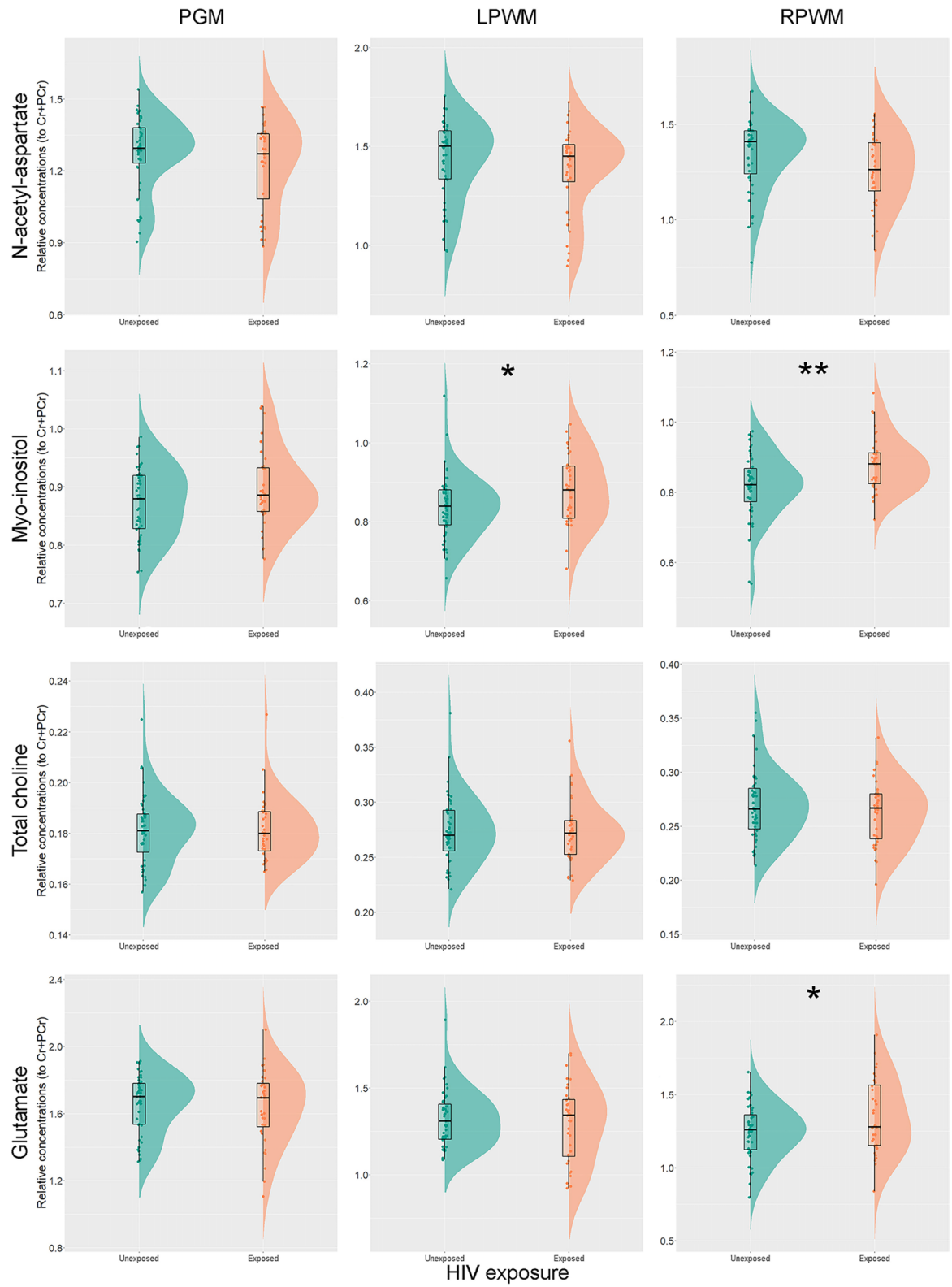

FIGURE 3 | Individual metabolite relative concentrations. Raincloud plots (54) showing individual metabolite relative concentrations to total creatine in the parietal grey matter (PGM), left parietal white matter (LPWM) and right parietal white matter (RPWM) in our complete-case cohort, according to HIV exposure. *p<0.05; **p<0.01.

Cr+PCr reach final, stable values within the first year of life (32). Therefore, a pattern of covarying Ins/Cr+PCr across brain regions at 2-3 years of age suggests neurometabolic development in CHEU may be influenced by underlying neuroinflammatory processes. Of note, maternal alcohol use during pregnancy did not substantially modify the results of the unadjusted analysis, despite its described association with lower glutamate concentrations in the parietal WM in neonates (50).

While there are no previous MRS reports of CHEU at this age, neurometabolic differences in this population have been reported in older children. Low absolute concentrations of creatine and phosphocreatine, n-acetyl-aspartate, total choline, and glutamate were found in the basal ganglia of a South African cohort of CHEU at age 9 years, compared to their unexposed peers, indicating possible neuronal damage (35). A longitudinal analysis of the same cohort found no interactions between age and HIV exposure when exploring neurometabolic development from 5 to 10 years of age (34). Further, at age 11 years, lower absolute concentrations of n-acetyl-aspartate were observed in frontal GM and peritrigonal WM of CHEU, suggesting possible axonal damage (36). Taken together, these results reflect the dynamic nature of neurometabolic development across child ages and brain regions, and the importance of analysing neurometabolites at different ages. However, children at older ages may have been exposed to additional sociodemographic and psychosocial risk factors that may impact their brain development adding a layer of complexity to the interpretation of results. Our study has the advantage of exploring neurometabolic development at a younger age, minimising the influence of socioenvironmental confounders.

Ins/Cr+PCr was significantly higher in left and right parietal WM of CHEU in our unadjusted analysis, and right parietal WM differences remained significant after adjusting for child age, child sex, and maternal alcohol use during pregnancy. WM may therefore be particularly sensitive to neuroinflammation from HIV exposure. Altered WM microstructural development has previously been reported in the right posterior corona radiata and the corticospinal tract of CHEU at age 7 years compared to CHU (31), and in neonates from the DCHS in the middle cerebellar peduncles (29) supporting our findings.

In addition to our main finding of higher parietal Ins/Cr+PCr in CHEU, we found differences in other metabolite ratios between groups. Glu/Cr+PCr levels were higher in the right parietal WM of CHEU in both unadjusted and adjusted analyses, compared to CHU. While covarying levels of Glu/Cr+PCr in WM were considered a pattern for neuronal function in our factor analysis, in the context of HIV exposure and neuroinflammation glial cells are primed and may fail to regulate glutamate. This has been demonstrated in patients with brain injuries or neuropsychiatric disorders, resulting in an unusual increase of this neurotransmitter in the extracellular space (55–57), which may also explain our results here. No results were modified after adjusting for GM percentage in voxels targeted at parietal WM in our sensitivity analyses, despite the presence of this confounder in the composition of such voxels.

Overall, our findings of increased Ins/Cr+PCr in the WM of CHEU add to the literature that HIV exposure may impact on WM development by affecting underlying neuroinflammatory processes. Animal model studies suggest that maternal immune activation induces exaggerated neuroinflammatory processes in offspring (19, 20). One of the main reported effects is microglial priming, where microglial cells become prone to produce an exaggerated response against second hits (18). Therefore, postnatal threats such as infections or environmental stressors, may elicit a neuroinflammatory overreaction in the young brain with long-term consequences (18–20). *In utero* priming of the immune system may take place in CHEU (21–23), and of note, inflammatory metabolite patterns of myo-inositol and total choline have been associated with cognitive impairment in adults (37, 38, 58) and children (35, 59) living with HIV.

Psychosocial variables may also play a key role in the neurometabolic development of CHEU. In LMICs studies, maternal depression and alcohol use during pregnancy have separately been associated with poorer cognitive outcomes in this population (8, 60). A recent US study linked maternal depression with decreased creatine and phosphocreatine, n-acetyl-aspartate, and total choline levels in the developing brain of HIV-unexposed foetuses (61) suggesting maternal immune activation may play a role (62). We found the impact of HIV exposure on Ins/Cr+PCr was independent of maternal depression and alcohol use in pregnancy. However, whether the neurobiological mechanisms underpinning these factors overlap with those derived from HIV exposure needs to be determined in larger samples. Separately, infant birthweight has been associated with maternal HIV infection (63). Although, studies are heterogeneous, suggesting the relationship between maternal HIV status and infant birthweight may vary across settings (51). Given birthweight may be influenced by maternal immune activation during pregnancy (64) and has been reported to impact children's performance in developmental assessments at 2 years of age (8), we examined infant birthweight in sensitivity analyses and found this did not modify our results.

HIV-specific factors have also been found to impact CHEU outcomes, including maternal CD4 and ART. In a sub-study of CHEU from the South African CHER cohort, lower CD4/CD8 ratio in infancy correlated to lower basal ganglia n-acetyl-aspartate and total choline levels at 5 years (65), lower total choline levels at 7 years, and lower myo-inositol levels at 9 years of age (35). The results suggest that an altered immune status in infancy may be associated with poorer neuronal and glial cell density in childhood. Since long-term ART exposure has been linked to mitochondrial toxicity in the brain (24, 66), MRS could also be used in CHEU to measure mitochondrial markers, such as lactate (32, 33). Although we were limited in our ability to examine maternal CD4 and ART in this sample, future studies may examine the relationship between these variables and neurometabolites in CHEU.

The strengths of our study include the use of a robust approach to study the effect of HIV exposure on neurometabolic development at 2-3 years of age, comparing a well-characterized

sample of CHEU to an appropriate control group with similar sociodemographic characteristics from a LMIC setting. Overall, our findings provide novel information about the neurobiological profile of young CHEU in a sub-Saharan African setting. We performed robust sensitivity analyses which did not substantially modify the results obtained in the adjusted logistic regression model. Furthermore, our cohort had a high prevalence of sociodemographic and psychosocial risk factors, comparable to other LMICs, and, all mothers living with HIV in our cohort received first-line triple ART, the majority with a fixed dose combination of efavirenz, emtricitabine, and tenofovir, implying our cohort may have generalisability for other CHEU populations across sub-Saharan Africa.

This study has some limitations to consider in the interpretation of our findings. First, MRS in very young paediatric subjects is technically challenging, since lack of motion is essential for successful data acquisition. As some data were lost due to children motion, the size of our complete-case cohort for analysis was substantially reduced, resulting in potential for underpowering of our analysis. However, sociodemographic characteristics were similar between the complete-case cohort and the full neuroimaging cohort, minimizing the likelihood of selection bias. This reduction in sample size meant we were unable to explore the association of maternal CD4 cell counts during pregnancy with child metabolite patterns, which needs to be investigated in future work. Second, our study design only included voxels placed in the parietal regions of the developing brain, so we are unable to draw conclusions about the presence of an inflammatory pattern in other brain areas of CHEU. Third, since WM is still under maturation in the developing brain (67), the tissue composition of voxels targeted at parietal WM may have included a proportion of GM. Hence, we cannot claim metabolite ratios obtained from these voxels purely belong to WM tissue. To mitigate this limitation, we ran sensitivity analyses for region-specific comparisons of individual metabolite ratios between groups, adjusting for GM percentage in voxels targeted at parietal WM, and found our results held. Fourth, although total creatine is well characterized and stable in the neurotypical brain during the first years of life (32, 33), low levels of this reference have been described in the peritrigonal WM in children living with HIV (36), compared to CHEU and CHU, and in subcortical brain regions in CHEU, compared to CHU (35). In contrast, higher creatine levels have been described in the parietal WM in adult subjects living with HIV, compared to uninfected peers (37). Therefore, although relative concentrations are commonly reported as they have the advantage of being less dependent on correction for relaxation and partial volume effects compared to absolute concentrations, the use of creatine and phosphocreatine as a reference in CHEU studies complicated interpretation as findings may reflect a change in the numerator or denominator. Similarly, the roles of metabolites in the developing brain, particularly n-acetyl-aspartate, remain to be fully established and Factor interpretations should be viewed with some caution. Lastly, we did not correct for multiple comparisons in our analyses, given the exploratory nature of our study and our use of factor analysis as a dimensionality-reduction method to reduce comparisons. Further work will be needed in larger sample sizes to replicate results.

In conclusion, our study presents the first results of the neurometabolic impact of HIV exposure in children from a LMIC setting during their first 2-3 years of life. We report differences in brain metabolite patterns between CHEU and CHU, showing an association of HIV exposure with an inflammatory pattern of elevated Ins/Cr+PCr in parietal brain regions. Our results are suggestive of neuroinflammatory processes in the developing brain of CHEU at this early age, which may be especially relevant in the parietal WM; whether this represents a potential target for specific neurodevelopmental interventions remains to be determined. Future work is needed to assess the longitudinal trajectories of neurometabolites in the population of CHEU, and to investigate associations with neurocognitive development and mechanisms underlying the inflammatory profile.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009; 525/2012 & 044/2017), by Stellenbosch University (N12/02/0002), and by the Western Cape Provincial Health Research committee (2011RP45). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CB-C: methodology, formal analysis and interpretation, visualization, writing – original draft, and review & editing. CW: conceptualization, investigation, data curation, supervision, and writing - review & editing. FR: methodology, formal analysis, supervision, and writing – review & editing. SS: investigation and writing – review & editing. KN: methodology and writing – review & editing. SJ: methodology and writing – review & editing. ARo: investigation and writing – review & editing. NH: project administration and writing – review & editing. ARe: formal analysis and writing – review & editing. HZ: conceptualization, methodology, resources, and writing - review & editing. DS: conceptualization, methodology, resources, and writing – review & editing. KD: conceptualization, methodology, investigation, resources, supervision, and writing – review & editing. All authors approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.800273/full#supplementary-material

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Supplementary Material

Supplementary Table 1 | Sociodemographic characteristics of the complete-case cohort of children included in the MRS analysis, compared to all children with MRS scans.

	Complete-case cohort (N = 83)	Original cohort (N = 156)	
	Mean (±SD) or n/N (%)	Mean (SD) or n/N (%)	p value
Child age at scan (in months)	33.99 (± 1.79)	33.96 (± 1.77)	0.71
Sex			0.91
Male	49/83 (59.04%)	91/156 (58.33%)	
Female	34/83 (40.96%)	65/156 (41.67%)	
Monthly household income (in ZAR)			0.70
< 1000	29/83 (34.94%)	48/156 (30.77 %)	
1000 - 5000	49/83 (59.04%)	95/156 (60.90 %)	
> 5000	5/83 (6.02%)	13/156 (8.33 %)	
Maternal education			0.96
Primary	6/83 (7.23%)	9/156 (5.77%)	
Some secondary	48/83 (57.83%)	92/156 (58.97%)	
Completed secondary	25/83 (30.12%)	46/156 (29.49%)	
Tertiary	4/83 (4.82%)	9/156 (5.77%)	
Maternal employment	18/83 (21.68%)	42/156 (26.92%)	0.37
Maternal relationship status (partnered)	36/82 (43.90%)	74/155 (47.74%)	0.57
Maternal age at delivery (in years)	27.49 (± 5.19)	27.94 (± 5.75)	0.68
Gestational age at delivery (in weeks)	38.75 (± 2.61)	38.79 (± 2.40)	0.99
Premature birth (< 37 weeks' gestation)	11/83 (13.25%)	21/156 (13.46%)	0.96
Birthweight (in g)	3088 (± 572.24)	3144 (± 562.67)	0.87
Nutritional conditions at 2 years old (based on WHO Z scores guidelines)			

Stunting (height-for-age Z-score < -2)	10/72 (13.88%)	28/137 (20.44%)	0.24
Underweight (weight-for-age Z-score < -2)	3/72 (4.16%)	8/137 (5.84%)	0.60
Wasting (weight-for-length Z-score < -2)	0/72 (0.00%)	1/137 (0.73%)	-
Maternal hospitalization during pregnancy	7/83 (8.43%)	10/154 (6.49%)	0.58
Maternal smoking during pregnancy	18/81 (22.22%)	29/151 (17.22)	0.59
Maternal alcohol use during pregnancy	13/81 (16.05%)	23/127 (18.11%)	0.70
Maternal depression during pregnancy	12/70 (17.14%)	31/129 (24.03%)	0.26
Exclusive breastfeeding duration (in months)	2.06 (± 1.84)	1.83 (± 1.79)	0.30
Maternal HIV status			0.87
Positive	36/83 (43.37%)	66/156 (42.31%)	
Negative	47/83 (56.63%)	90/156 (57.69%)	

Data are mean (± SD) or n/N (%). Percentages calculated out of available data. Continuous data was assessed for normality using Shapiro-Wilk tests. Comparisons between CHEU and CHU were made using *t*-tests or Wilcoxon tests for normally and non-normally distributed continuous data, respectively, and X^2 tests with Yates correction for categorical data.

Missing data: maternal relationship status (N = 1 in the complete-case cohort, N = 1 in the original cohort); nutritional conditions at 2 years old (N = 11 in the complete-case cohort, N = 19 in the original cohort); maternal hospitalization during pregnancy (N = 2 in the original cohort); maternal smoking during pregnancy (N = 2 in the complete-case cohort, N = 5 in the original cohort); maternal alcohol use during pregnancy (N = 2 in the complete-case cohort, N = 29 in the original cohort); maternal depression during pregnancy (N = 13 in the complete-case cohort, N = 27 in the original cohort).

Supplementary Table 2 | Sensitivity analyses for maternal age of delivery, maternal depression during pregnancy, and infant birthweight.

	Sensitivity analysis for maternal age at delivery*			Sensitivity analysis for maternal depression**			Sensitivity analysis for infant birthweight***		
	OR	Confidence interval (95%)	P value	OR	Confidence interval (95%)	P value	OR	Confidence interval (95%)	P value
Factor 1 (NAA)	0.62	0.34 – 1.00	0.09	0.63	0.35 – 1.08	0.10	0.76	0.03 – 2.97	0.24
Factor 2 (Ins)	**1.82**	**1.17 – 3.02**	**0.012**	**1.65**	**1.04 – 2.82**	**0.045**	**1.56**	**1.05 – 2.44**	**0.036**
Factor 3 (GPC+PCh)	0.94	0.45 – 1.88	0.86	1.10	0.54 – 2.31	0.79	0.79	0.39 – 1.50	0.47
Factor 4 (Glu)	1.26	0.69 – 2.34	0.45	1.14	0.59 – 2.27	0.69	1.46	0.82 – 2.68	0.20

Logistic regression analysis of factor scores as predictors for HIV exposure. Odds ratios (OR) greater than 1 indicate an increased likelihood of association between a specific metabolite pattern and HIV exposure. Bold data represents values belonging to statistically significant associations ($p<0.05$). *Adjusted for child age, child sex, maternal alcohol use during pregnancy and maternal age at delivery. **Adjusted for child age, child sex, maternal alcohol use during pregnancy and maternal depression during pregnancy. ***Adjusted for child age, child sex, maternal alcohol use during pregnancy and infant birthweight.

NAA: metabolite pattern dominated by n-acetyl-aspartate ratios; Ins: metabolite pattern dominated by myo-inositol ratios; GPC+PCh: metabolite pattern dominated by total choline (glycerophosphocholine + phosphocholine) ratios; Glu: metabolite pattern dominated by glutamate ratios.

Supplementary Table 3 | Comparison of region-specific metabolite ratios between CHEU and CHU groups.

	Parietal grey matter						Left parietal white matter						Right parietal white matter					
	Unadjusted			Adjusted*			Unadjusted			Adjusted*			Unadjusted			Adjusted*		
Metabolite ratios	**β**	**Robust SE**	**p-value**	**β**	**Robust SE**	**p-value**	**β**	**Robust SE**	**p-value**	**β**	**Robust SE**	**p-value**	**β**	**Robust SE**	**p-value**	**β**	**Robust SE**	**p-value**
NAA/Cr+PCr	-0.140	0.037	0.21	-0.174	0.040	0.15	-0.117	0.046	0.29	-0.109	0.050	0.36	-0.212	0.041	0.054	-0.231	0.036	0.07
Ins/Cr+PCr	0.091	0.016	0.42	0.112	0.019	0.34	**0.246**	**0.018**	**0.025**	0.223	0.020	0.066	**0.335**	**0.018**	**0.001**	**0.329**	**0.021**	**0.004**
GPC+PCh/Cr+PCr	0.076	0.003	0.50	0.065	0.003	0.58	-0.013	0.006	0.90	-0.060	0.005	0.58	-0.107	0.006	0.328	-0.143	0.006	0.22
Glu/Cr+PCr	-0.053	0.044	0.63	-0.026	0.047	0.83	-0.073	0.043	0.52	-0.043	0.045	0.71	**0.241**	**0.048**	**0.034**	**0.287**	**0.051**	**0.015**

In unadjusted and adjusted analyses, comparisons between CHEU (N = 36) and CHU (N = 47; reference group) were made using linear regression analyses with robust standard errors. *Adjusted for child age, child sex, and maternal alcohol use during pregnancy. Bold data represents values belonging to statistically significant associations ($p<0.05$).

β: standardized coefficient; SE: standard error; NAA: n-acetyl-aspartate; Ins: myo-inositol; GPC+PCh: total choline (glycerophosphocholine + phosphocholine); Glu: glutamate; /Cr+PCr: relative to creatine + phosphocreatine.