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Autism outcomes and neurobehavioural markers in young children born to mothers with HIV in Kenya: a protocol for the Alama project

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ABSTRACT

Introduction The over 14 million African children who are HIV-exposed but uninfected (CHEU) are at risk for poor health outcomes, including neurodevelopmental conditions such as autism; however, no study to date has examined autism in CHEU in Africa, where the vast majority of these children live. Scalable diagnostic and neurobehavioural tools, including powerful, low-cost approaches such as eye-tracking, for detection and study of mechanistic neural processes are necessary to advance autism research in these settings. The objective of this study is to examine autism diagnostic outcomes and eye-tracking biomarkers in relation to CHEU while at the same time building capacity for neuro-health research in Kenya.

Methods and analysis This study will leverage a longitudinally assessed cohort of CHEU and children who are HIV-unexposed and uninfected (CHUU) with well characterised HIV-related and contextual exposures. We will first determine and compare autism diagnostic outcomes between young CHEU and CHUU across a large cohort (n=850) of Kenyan children using research-grade autism assessment tools, and, second, determine whether neurobehavioural eye-tracking markers predict autism outcomes across this cohort.

Ethics and dissemination Human subjects approvals have been obtained from Moi University Institutional Review and Ethics Committee (IREC; IREC/909/2024; Approval #0004835), Kenya's National Commission for Science, Technology and Innovation (NACOSTI; Reference #NACOSTI/P/25/415028), the Institutional Review Board of the Indiana University School of Medicine (Protocol #23171), with reliance agreements executed with Purdue University and Boston University. Dissemination of findings will occur through multiple channels within the research and clinical community, including peer-reviewed journal publications and conference abstracts and presentations. As part of capacity building efforts, the research team will also communicate study results to policy makers, the lay public and other health systems involved in the care of young children with disabilities via study-hosted workshops and conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Large longitudinal cohort leveraging rich, prospectively collected prenatal, perinatal and early postnatal parent study data critical for confounding control and mediation tests.
⇒ Blindly administered, gold-standard autism diagnostic battery plus culturally adapted developmental testing.
⇒ Rigorous eye-tracking battery with standardised setup, calibration, quality control metrics and multiple tasks positions the study to evaluate several scalable autism biomarkers.
⇒ Actual rates of autism may be lower than our conservative estimates and could limit power for analysis of categorical outcomes.
⇒ While demographic, environmental and psychosocial information has been collected at several prenatal, perinatal and postnatal timepoints, residual confounding remains possible.

INTRODUCTION

With the success of population health efforts to scale use of antiretroviral therapy (ART) for those living with HIV, there has been a substantial reduction in vertical (ie, mother-to-child) transmission rates.[1] However, the number of uninfected infants exposed to HIV through their HIV-infected mothers is rising. Currently, 90% of the estimated 14.8 million children who are HIV-exposed and uninfected (CHEU) live in Africa.[2] Exposure to maternal HIV and ART and their sequelae, including adverse birth outcomes,[3] growth problems[4 5] and increased infectious morbidity and mortality,[6] places CHEU at greater risk for poor health.[7]

Despite advances in understanding the health of CHEU,[1 8] neurodevelopmental outcomes have not been well studied.[7] Previous research has shown that young CHEU demonstrate delays in cognitive and motor development.[9 10] Several small studies conducted in high-income countries (HIC) suggest that CHEU, compared

with children who are HIV unexposed and uninfected (CHUU), may have a higher prevalence of neurodevelopmental conditions, including autism.[11–13] Autism is a neurodevelopmental disability with biological differences emerging in the prenatal period that can be reliably diagnosed in the first two to three years of life based on impairments in social communication and the presence of restricted and repetitive behaviours.[14] Based on several small studies conducted in HIC, it has been hypothesised that CHEU are at elevated risk for autism due to prenatal and perinatal HIV and ART exposures associated with preterm birth and low birth weight,[15 16] iron-deficiency anaemia,[17] breastfeeding practices[1] and maternal[18 19] and child[20 21] infectious morbidity. These HIV-related and contextual risks potentially mediate HIV/ART exposure and autism outcomes[22–27] but have not been examined in CHEU living in low- and middle-income countries (LMIC).

There is virtually no data on autism in most LMIC,[28–30] and no study to date has examined autism in CHEU in LMIC where the vast majority of the world's children live. Investigating autism within CHEU has the high potential to shed light on critical mechanisms underlying the development of the condition. For example, CHEU are exposed to maternal HIV and associated maternal inflammatory response in the prenatal period[31] and demonstrate altered postnatal immune regulation and inflammation,[32] both of which may confer increased likelihood for the development of autism.[28 33–35] Rigorous research on autism in CHEU living in LMIC can help to identify mediating risks on the causal pathway between HIV/ART exposure and autism outcome[36 37] and further inform the selection of modifiable targets for precision detection and intervention strategies necessary to improve autism outcomes in this setting.[28–30]

Research on autism in CHEU cannot be advanced without rigorous methods to accurately diagnose and characterise behavioural phenotypes. Standardised autism diagnostic tools have primarily been developed and validated in HIC with non-diverse populations, are proprietary and costly, and require high levels of training resulting in limited utility in the context of research in LMIC.[35 38–40] Easy-to-administer, culturally appropriate, open-source approaches that harness technology and task sharing with non-professionals are key to improving autism detection in these settings.[29] Objective, scalable neurobehavioural markers could substantially advance autism diagnosis in LMICs that lack neurodevelopmental experts.[41–43] Eye tracking has demonstrated utility as a non-invasive, low-cost, feasible and effective method for identifying autism in young children.[44–46] Prior research has demonstrated that a series of eye-tracking markers reliably differentiates young children with autism and can be applied in resource-constrained settings in the US.[47] These eye-tracking markers assay neurocognitive processes such as social[48–52] and non-social[53–55] attention and neuromodulator function[56–61] to elucidate early behavioural differences and their neural underpinnings in young children with autism. For example, pupillary light reflex, associated with the neurotransmitter acetylcholine,[58] is larger in 9- to 10-month-old infants at elevated likelihood for autism[59 62] and is associated with later autism diagnosis and symptom severity.[59] Tonic or resting pupil size, an indirect index of locus-coeruleus/norepinephrine activation,[56] has been shown to be larger in two independent samples.[57] To date, this research has only been conducted in HIC but represents a promising scalable method for autism detection, diagnosis and prediction of neurodevelopmental outcomes in LMIC.[63]

To achieve improved health outcomes in LMIC, a key research priority must include capacity building in state-of-the-science diagnostic and neurobehavioural methods for autism detection.[35 38–40] The investigative team – representing interdisciplinary clinicians and researchers from both Kenya and the US - has been studying neurodevelopmental outcomes in CHEU and building neuroscience research capacity within Kenya, an LMIC with a high HIV burden but limited resources to conduct rigorous autism research. This foundational work has ideally positioned the team to now focus on examining autism outcomes in CHEU, a critically important next step given that early detection and intervention improve developmental outcomes and reduce family stress and long-term care costs.[14]

METHODS AND ANALYSIS

Aims and hypotheses

This study, the Alama Project ("alama"="marker" in Kiswahili), directly addresses critical gaps in our understanding of autism outcomes in the growing population of CHEU in LMIC. The overall objective is to examine autism diagnostic outcome and to validate eye-tracking markers in CHEU while synergistically building capacity in state-of-the-science autism research methodologies in Kenya. The aims of this study are:

Aim 1: compare autism diagnostic outcomes between Kenyan CHEU and CHUU. We hypothesise that CHEU will have higher rates of autism compared with CHUU (1.1), and HIV-related and contextual risk factors will mediate the effect of HIV/ART exposure on autism outcome (1.2).

Aim 2: determine whether neurobehavioural eye-tracking markers predict autism outcome in CHEU and CHUU in Kenya. We hypothesise that eye-tracking markers will predict autism outcome (2.1), and there will be a significant association between eye-tracking markers and distinct HIV-related and contextual risks (2.2).

This prospective cohort study will be the first to examine autism outcomes in CHEU and to determine the validity of eye-tracking markers as a potential scalable diagnostic tool in LMIC. Investigating autism in CHEU has the high potential to identify

important mediating risks between HIV/ART exposure and autism outcome, which represents a critical step in elucidating the neurodevelopmental impact of in-utero HIV and ART exposure and has the potential to inform the selection of modifiable targets for precision detection and intervention strategies necessary to mitigate deleterious child health outcomes.

Study setting

The proposed research will be conducted within the Academic Model Providing Access to Care (AMPATH) programme, a >30 year collaboration between Moi University School of Medicine and Moi Teaching and Referral Hospital in western Kenya, and a consortium of North American academic medical centres led by Indiana University School of Medicine (Indianapolis, IN, USA). Since 2001, AMPATH has led one of the largest HIV prevention and treatment programmes in Africa, bringing the tripartite mission of clinical service, education and research to solve challenges of delivering population-based healthcare in a low-resource setting.[64 65]

Study sample

This prospective cohort study will be built on two connected parent studies situated within AMPATH Kenya: (1) Measuring adverse pregnancy and newborn congenital outcomes (MANGO; clinicaltrials.gov #NCT04405700) and (2) Tabiri study: MANGO follows a cohort of 1600 pregnant women through delivery to examine the impact of ART on pregnancy outcomes.[66] The Tabiri Study recruits a subset of CHEU and CHUU children born to mothers enrolled in MANGO and follows them through 24 months of age to examine neurodevelopmental outcomes.[67] Across MANGO and Tabiri, rich data on birth and prenatal history, maternal and child infectious morbidity, biological, psychosocial and sociodemographic factors are collected. The present study leverages specific data from these parent studies based on empirically derived hypotheses regarding autism outcomes. For the Alama Project, we will attempt to recruit and enrol all children that completed the Tabiri Study to examine autism diagnostic outcomes and eye-tracking markers during the 24-month to 48-month period (see figure 1).

The current study will examine autism diagnostic outcomes and eye-tracking biomarkers in n=850 children; n=425 CHEU, defined by being born to a mother diagnosed with HIV before or during pregnancy, and n=425 CHUU, defined as being born to a mother without a known HIV diagnosis before or during pregnancy. Inclusion criteria for children are: (1) Enrolment in the Tabiri study, (2) Age 24 to 48 months at the time of recruitment and (3) Having a primary caregiver >18 years old who is fluent and can provide consent in English or Kiswahili. There will be no specific exclusion criteria. We expect that there will be a small number of children who test

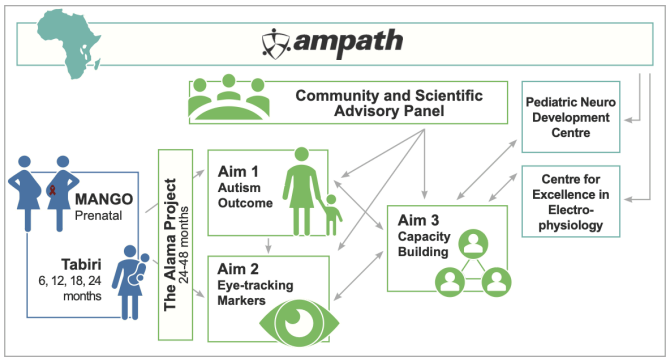

Figure 1 Study overview. MANGO, measuring adverse pregnancy and newborn congenital outcomes.

positive for HIV (CHIV+; tested at 24-month Tabiri visit); these children will not be excluded from the present study (see Data Analysis for specific plan).

Study design and procedures

Children will be recruited, consented and enrolled at the Tabiri 24-month study visit or thereafter if the visit has been completed prior to Alama study start. Data on exposures, mediators and confounders will be gathered from MANGO and Tabiri. To ensure unbiased diagnostic decision making, study personnel involved in evaluations will be blinded to participant group status (ie, CHEU, CHUU). The Alama Project will consist of a single study visit. The research evaluation, estimated to take 2–4 hours to complete, will include administration of caregiver and child clinical/diagnostic and eye-tracking measures. As participants are toddlers and very young children, these measures will be flexibly administered to accommodate the needs of children and their caregivers. Opportunities for breaks, play and naps will be available for children as needed. Caregivers will be provided with verbal and written feedback about their child's evaluation, including developmental level and diagnosis, at the end of the research evaluation. The investigative team has developed educational resources and an intervention referral plan for children with identified medical conditions, neurodevelopmental delays and autism. Families will be compensated $20/2500.00 Kenya Shillings for participation in the research evaluation and $10/KShs 1250.00 for travel to the study; this compensation is consistent with well-accepted research remuneration standards in Kenya.

Study measures

Our longitudinal, multi-method and multi-informant assessment strategy includes prospectively gathered primary exposures, contextual risks and potential confounders as well as clinical/diagnostic and eye-tracking measures.

Primary exposures

We will obtain the following primary exposure data from the MANGO study: maternal HIV viral load during

pregnancy, timing of initiation of ART (before or during pregnancy), maternal ART regimen categorisation (dolutegravir-based, other antiretroviral (ARV)-based or none) and post partum infant ARV prophylaxis regimen. We will obtain the following primary exposure data from the Tabiri study: plasma dolutegravir and efavirenz levels collected at 6 and 24 months post partum from all breastfeeding CHEU within an hour of a feed, and 3–4 hours after a maternal ART dose. These exposures will be absent in CHUU.

Mediating risk factors

We will obtain data on mediating risk factors from both MANGO and Tabiri studies.

Birth outcomes and in utero exposures (MANGO)

Weeks of gestation at birth (ie, by prenatal ultrasound or estimated from last menstrual period; <37 weeks, ≥37 weeks), birth weight (<2500 gm vs ≥2500 gm) and reported maternal infections during pregnancy (yes vs no).

Inflammatory marker testing (Tabiri)

Inflammatory biomarkers are measured by the AMPATH reference lab from cryopreserved plasma samples taken at 6 and 24 months of age. C-reactive protein, ferritin, fibrinogen, sCD163, sCD14, IL-22, IL-2R and TWEAK are measured with ELISA assays. D-dimer and inflammatory cytokines, IFNα, IFNγ, IL-1α, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 p70, IL-15, IL-17A, IL-21, Tau and TNFα, are quantified using a multiplex assay on Luminex. We will use principal components analysis to examine patterns of markers in CHEU and CHUU.[68]

Iron deficiency anaemia (IDA) testing (Tabiri)

At 6 and 24 months of age, children visit the AMPATH Reference Lab for phlebotomy. A complete blood count is performed. For breastfeeding CHEU, their remaining sample was processed in cryopreserved plasma, shipped and tested at Indiana University to obtain ferritin levels. We will apply WHO guidelines for definitions of iron deficiency and anaemia (IDA), while accounting for the setting's elevation of 1500–2000 m above sea level. IDA will be defined by a haemoglobin level of <10.5 mg/L (cut-off for moderate anaemia)[69] and ferritin level of <12 µg/L.[70]

Breastfeeding (Tabiri)

Breastfeeding frequency and duration are measured at baseline, 6, 12, 18 and 24 months. Options for breastfeeding incidence will be: 0, 25%, 50%, 75% or 100% of feeds at each time point. Information regarding nutritional diversity, including cow's milk consumption, is also obtained.

Child infectious morbidity (Tabiri)

INFORM-AIDS FREE is a harmonised infrastructure for monitoring health outcomes of children born to mothers living with HIV. With the input of expert panel consensus and technical working groups, outcome domains and data collection forms on a variety of outcomes, including infectious morbidity, are available. INFORM-AIDS Free project instruments are used to capture a spectrum of child infectious morbidity data, including diarrhoea, pneumonia, malaria, meningitis, measles and tuberculosis.

Confounders (Tabiri)

Maternal age and education, maternal alcohol consumption during pregnancy is measured using the WHO 8-question survey,[71] and poverty risk is measured using the Poverty Probability Index.[72]

Residual confounders by additional measures (MANGO/Tabiri)

Various biological (eg, APGAR scores, anthropometrics, lead levels) and psychosocial (eg, maternal depression, HIV-related stigma, child stimulation/punishment) variables are collected as part of the MANGO and Tabiri studies. These measures will allow us to examine residual confounding that may impact the validity of our findings.

Clinical/diagnostic measures (Alama)

In the present study, we have selected a battery of clinical and diagnostic measures widely used in neurodevelopmental and autism research.

Autism Diagnostic Observation Schedule, second edition (ADOS-2)[73]

The ADOS-2 is a semi-structured standardised observational measure of social communication, play and restricted and repetitive behaviours for children aged 12 months and older with varying developmental and language levels. It is considered the gold standard for autism diagnosis, with excellent psychometric properties, including sensitivity and specificity. Algorithm scores yield a diagnostic classification (ie, Autism, Autism Spectrum or Non-Spectrum) and a Calibrated Severity Score (CSS) is generated to provide a measure of autism symptom severity. ADOS-2 categorical classification will be used to inform best estimate autism diagnosis (primary outcome); CSS scores will be used as a secondary continuous (dimensional) measure of autism symptom severity.

Social Responsiveness Scale, second edition preschool (SRS-2 preschool)[74]

The SRS-2 Preschool Form is a caregiver-report measure of presence and severity of social communication and restricted and repetitive behaviours associated with autism. For the present study, the SRS-2 will be delivered via interview (estimated to take 20 min). T-scores across five treatment subscales, Total Score and DSM-5 Compatible Scales are generated. Scores will be used to quantify children's dimensional autism traits. The tool has demonstrated excellent psychometric properties[74] and has been translated, adapted and validated for use in several other countries[75–77] (including for use in children as young as 24 months).[76] SRS-2 scores will be used as a secondary continuous (dimensional) measure of autism symptom severity.

Toddler Autism Symptom Inventory (TASI)[78]

The TASI is a brief semi-structured interview designed for 12- to 36-month-old children. The TASI consists of 40 items that assess DSM-5 autism diagnostic criteria in the domains of social communication, repetitive behaviours and sensory processing. Each item is scored as present or absent. The TASI provides an empirically derived cut-off score indicating likelihood of autism, which has demonstrated strong ability to indicate autism risk. TASI classification will be used to inform best estimate autism diagnosis (primary outcome); Total score will be used as a secondary continuous (dimensional) measure of autism symptom severity.

Bayley Scales of Infant and Toddler Development, fourth edition (Bayley-4)[79]

The Bayley-4 is a comprehensive, norm-referenced assessment of child development across the domains of cognitive, language, and fine and gross motor skills for infants and children 16–42 months of age. The Bayley-4 takes 30–70 min to administer and has been culturally adapted for use in western Kenya.[80] The Bayley-4 is administered at the 24-month Tabiri research visit. We will repeat Bayley-4 administration only in cases where ≥6 months have elapsed since administration. Our primary use of Bayley-4 scores will be in making differential diagnostic decisions between developmental delay and autism as part of clinical best estimate diagnosis; it will also be used in characterisation of phenotypic differences between autism and non-autism outcomes. We will calculate developmental quotients (DQ; [age equivalent/chronological age]×100) for each subscale.[81]

Primary outcome

Best-estimate autism diagnosis will be defined by categorical autism diagnosis (ie, autism presence or absence). Following well-accepted procedures,[82] best estimate autism diagnosis will be made by Kenyan study team clinicians trained to autism research diagnosis reliability based on synthesising all research evaluation data, including ADOS-2, TASI, SRS-2 and Bayley-4, and determining whether DSM-5 autism criteria are met (ie, by completion of a DSM-5 checklist).

Training, reliability and quality control for clinical and diagnostic procedures

Standardisation of assessment procedures, study personnel training and maintenance of reliability and quality control will be overseen by study team members. This includes experts in autism diagnosis and clinical phenotyping (ie, Certified Independent ADOS-2 trainers) with expertise in training personnel in autism diagnostic assessment and overseeing assessment fidelity and quality control procedures on multi-site projects requiring strict adherence to standardised clinical assessment batteries. Similarly, team members with expertise in Bayley-4 training and quality control for NIH-funded global health studies will oversee Bayley-4 training and quality control. Ten per cent of all research evaluations will be selected randomly to conduct reliability assessments on each measure and autism outcome.

Eye-tracking measures (Alama)

We have selected a battery of eye-tracking neurobehavioural markers previously shown to be sensitive to autism outcomes (table 1).[46 55 59 83 84] We will adapt our previous paradigms to include culturally appropriate images and videos. These eye-tracking markers assay neurocognitive processes such as social engagement,[48–52] non-social attention,[53–55] neuromodulator function[56–61] and basic oculomotor metrics[83 84] to elucidate early behavioural differences and their neural underpinnings in young children with autism. An SR Research EyeLink Portable Duo remote ET system measures eye movements and pupil diameter (500 Hz). Five tasks will be administered over 10–12 min. To ensure rigorous and replicable data acquisition, the child's positioning and environmental setup will be standardised and quality control metrics will be gathered (see table 1). Prior to the eye-tracking battery, children will complete a five-point calibration/validation procedure while viewing an animated cartoon with sounds. Drift check and correction will be conducted prior to each task.

Sample size estimation

Power calculations were performed with Power Analysis and Sample Size Software 2023[85] based on a conservatively estimated 20% recruitment attrition rate from the final Tabiri Study sample completing 24-month visits (n=850) yielding a total sample of n=680 (n=340 CHEU; n=340 CHUU). Estimations of autism outcome in CHUU and CHEU are 2% and 10%, respectively. CHUU estimates are based on previously reported conservative global prevalence estimates of autism.[86] CHEU estimates are conservatively based on (1) Previous studies suggesting that 6[13] to 30%[12] of CHEU have autism and (2) Preliminary M-CHAT (autism screening) data from the Tabiri study that shows that 29% of CHEU and 19% of CHUU in our sample screen positive for autism risk at 24 months. For Hypothesis 1.1, a sample of 680 will provide 96% power for a two-sided Wald test in logistic regression to detect a difference between 2% versus 10% in anticipated autism diagnosis rate between CHEU and CHUU. For Hypothesis 1.2, a sample of 680 will provide 85% power to detect a mediation effect for continuous mediators in logistic regression. If outcomes and covariates are missing at substantial rates, we will consider using the state-of-the-art resampling-based multiple imputation method.[87 88]

Data analysis plan

Hypothesis 1.1: CHEU will have higher rates of autism compared with CHUU

We will conduct binary logistic regression to test the direct effect of HIV and ART exposure on autism outcome (ie, whether CHEU/CHUU groups differ on rate of autism),

Table 1 Eye-tracking biomarker battery and quality control measures

Task/measure	Description
Non-social preference	*Rationale*: reliable differences in attention to non-social information are present in autistic children.[46 49 50] *Protocol*: an adapted GeoPref test.[46 49 50] *Measures:* % looking at non-social compared with looking at social/non-social videos.
Attentional disengagement	*Rationale*: infants later diagnosed with autism show less efficient attentional disengagement.[53–55] *Protocol*: a gap-overlap test.[55] *Data measures:* saccadic latency and proportion of no-shift trials for gap versus overlap condition.
Pupillary light reflex (PLR)	*Rationale*: PLR amplitude and latency differences are present in infants later diagnosed with autism.[59 62] *Protocol*: PLR paradigm similar to Nyström *et al*.[59] *Measures:* PLR latency and amplitude.
Resting eye-tracking	*Rationale*: differences in tonic pupil size[57 92 93] and oculomotor measures[83 84] have been shown in autistic toddlers. *Protocol*: similar to Anderson *et al*.[57] *Measures:* resting pupil diameter and oculomotor metrics.
Passive visual exploration	*Rationale*: both fixation duration[84] and saccade amplitude[83] during video watching differ in infants and children with autism. *Protocol*: four 30 s animated cartoons of nursery rhymes. *Measures:* oculomotor metrics.
Sample variability	*Rationale:* differences in data quality may impact eye-movement measures.[94 95] *Measures:* sample-to-sample variability (precision).
Fixation drift	*Rationale:* differences in data quality may impact eye-movement measures.[94 95] *Measures:* average fixation drift (accuracy).
Number of valid biomarkers	*Rationale:* a sufficient amount of data is required to ensure a reliable measurement of each biomarker. *Measure:* the number of biomarkers meeting the paradigm-specific minimum valid data criterion.

adjusted for theoretically important confounding covariates (maternal age, education, SES, alcohol use). Maximum-likelihood estimated ORs, their 95% CIs and p values will be calculated (see figure 2 for conceptual model).

Hypothesis 1.2: HIV-related and contextual risk factors will mediate the effect of HIV/ART exposure on autism outcome

MPLUS software[89] will be used to conduct a mediation analysis[90] with the INDIRECT option and logit link (ie, logistic regression framework), using two-sided tests (0.05 alpha) of the indirect, direct, and total effects in a path model (ie, using observed, not latent variables). The proportion mediated will be calculated as the ratio of the indirect effect to the total effect, which indicates the proportion of the total effect that is explained or accounted for by the mediator. Most mediators will be continuous; we will also explore dichotomised mediators as appropriate. A separate model will be conducted for each mediator. The models will be adjusted for theoretically important confounding covariates (maternal age, education, SES, alcohol use). ORs, their 95% CIs and p values will be reported.

Hypothesis 2.1: eye-tracking markers will predict autism outcome

A series of binary logistic regressions will be conducted with eye-tracking metrics as continuous independent variables and autism diagnosis as the binary outcome. Bivariable models will be created first. Then, a multivariable model will be built using the lasso logistic method[91] to select the combination of eye-tracking markers that predict autism. The overall discrimination of the model will be captured using the c-statistic. ORs, their 95% CIs and p values will be calculated for each predictor.

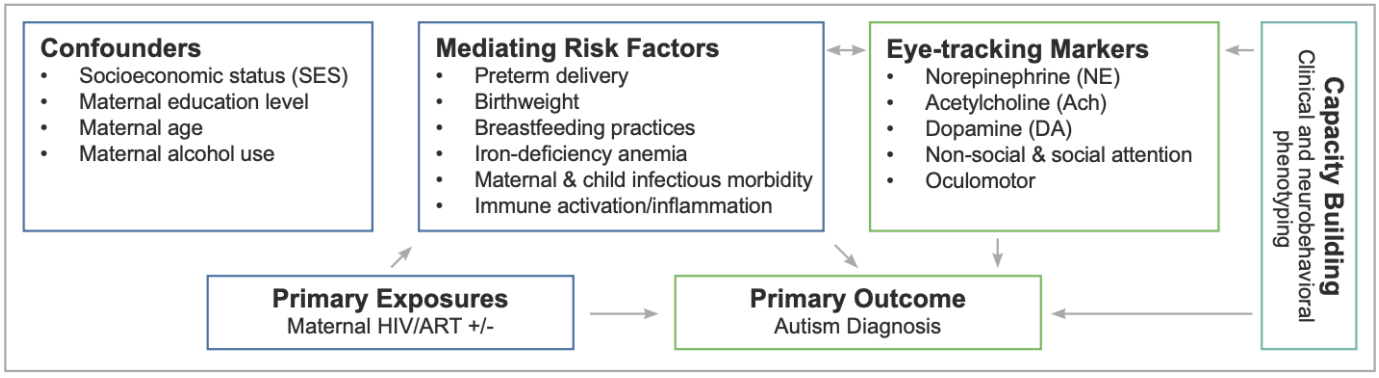

Figure 2 Conceptual model of study. ART, antiretroviral therapy.

Hypothesis 2.2: there will be a significant association between eye-tracking markers and distinct HIV-related and contextual risks

For markers that are significant predictors of autism, we will examine association with HIV-related and contextual risks to investigate potential mechanistic links between risk factors and eye-tracking metrics. We will use the Pearson correlation coefficient to compare continuous eye-tracking metrics and risk factors, and the Pearson χ^2 test to compare continuous eye-tracking metrics and categorical risk factors.

Given that we expect the number of CHIV+ to be small, it is unlikely that we will have adequate sample size to conduct a rigorous comparative analysis between CHIV+, CHEU and CHUU. We will conduct exploratory descriptive analyses to serve as preliminary data for a future study.

Capacity building

Research on autism in CHEU cannot be advanced without rigorous methods to accurately diagnose and characterise children. As such, a key priority for research capacity building is developing and validating standardised diagnostic tools and eye-tracking markers to identify children with autism in LMIC.[35 38–40] As part of this study, the research activities of Aims 1 and 2 will be integrated with broad capacity building and infrastructure development. The study team will deploy a tiered programme of intensive training and mentorship with the goal of building global scientific collaboration and upskilling a clinical and research workforce with expertise, tools and resources. Tiered activities will include development and deployment of a virtual repository of training seminars, annual workshops, mentored research projects and conferences to disseminate research findings and further catalyse collaboration. These activities will flexibly support options for self-guided, hands-on experiential and mentored research training with outcomes ranging from acquisition of foundational knowledge to achievement of research independence in novel methodologies. This approach will permit those with diverse research interests (eg, HIV and other infectious diseases) to apply training and tools toward their own areas of expertise. This work will culminate in a global collaborative well prepared to conduct neuro-health research that will drive developments in science, healthcare and policy.

Public involvement

The study team will engage a diverse panel of community and scientific advisors, including Kenyan parents of children with autism, Kenyan clinicians and researchers, and global health equity and neuroscience leaders at all stages of the research and capacity-building activities. We have intentionally designed an *integrated* community and scientific panel so that our research and capacity building will benefit from the constructive interaction of lived experiences, perspectives and areas of expertise offered by the panel members and to ensure rigour and relevance to the Kenyan culture and setting.

The role of the panel will include advising the research team on: (1) Study design and direction to ensure research meets community needs and LMIC context, (2) Adaptation and implementation of diagnostic and eye-tracking measures, (3) Interpretation and dissemination of findings and prioritisation and design of follow-up studies and (4) Community-academic partnership in capacity-building activities to ensure feasibility, relevance and sustainability of activities in LMIC context.

ETHICS AND DISSEMINATION

Consent and information provision

Human subjects approvals have been obtained from Moi University and Moi Teaching and Referral Hospital Institutional Review and Ethics Committee (IREC; IREC/909/2024; Approval #0004835), Kenya's National Commission for Science, Technology and Innovation (NACOSTI; Reference #NACOSTI/P/25/415028) and the Institutional Review Board of the Indiana University School of Medicine (Protocol #23171) with reliance agreements executed with Purdue University and Boston University. The study will be conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human participants and the Declaration of Helsinki. This study carries minimal risk to the children and their caregivers. All caregivers will provide written informed consent; however, assent will not be obtained from children due to their young age.

Dissemination plan

Timely dissemination of findings is important, and there is often a substantial lag between the time a journal article is published and when the knowledge reaches front-line practitioners and decision makers. Therefore, the research team will coordinate dissemination of findings through a variety of channels within the research and clinical community, including conference abstracts and presentations. The research team will publish study results in scholarly, peer-reviewed journals within the medical and public health fields. In addition to academic stakeholders, the research team will communicate study findings to policymakers, the lay public and other health systems involved in the care of young children with disabilities.

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Contributors EO assisted with study conceptualisation and will assist with clinical case review, data collection and analysis efforts. He is the Moi University MPI for this study. BK co-led study conceptualisation and will lead all eye-tracking aspects of the study. He is the Purdue University MPI. MSM is the PI of the Tabiri Study and assisted with study conceptualisation and data sharing efforts. POM assisted with study conceptualisation and developed the statistical analysis plan. RMJ assisted with study conceptualisation, clinical training and reliability. AK will lead the laboratory testing in Kenya. S-YY will assist with data analysis. JGC assisted with study conceptualisation and with MANGO data sharing efforts. CS will assist with clinical case review and capacity-building efforts. JB will assist with capacity-building efforts. RMK co-led study conceptualisation and protocol writing and will lead all aspects of the study. She is contacting MPI. All authors assisted with protocol writing or reviewing. RMK is responsible for the overall content as guarantor.

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