WASH Benefits Environmental Enteric Dysfunction and Neurodevelopment Analysis Plan

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1. Scientific background and rationale

An estimated 249.4 million children under 5 are at risk for poor development, amounting to almost half of children in low- and middle-income countries (LMICs).[1] Potential consequences of not reaching one's full developmental potential include lower academic achievement, reduced adult economic productivity, and the intergenerational transmission of poverty.[2–4] Poor child development is thought to be caused in part by malnutrition, lack of stimulating environment, and infection and is marked by deficits in social-emotional and cognitive functions.[1] However, these factors alone do not account for all of poor neurodevelopment; thus, it is critical to improve our understanding of other determinants. A child's first 1,000 days (from conception to age 2) is a critical epoch when the brain is more sensitive to environmental conditions and can impact higher order behavior.[2,3] Early life environmental exposures during the postnatal period can have long-lasting implications on a child's social and behavioral development.[5]

One emerging factor that could potentially contribute to poor child development in LMICs is a condition called environmental enteric dysfunction (EED). EED is characterized by inflammation, reduced absorptive capacity of nutrients, and reduced barrier function of the small intestine and is thought to be associated with poor physical growth in infancy and childhood.[6] Nutritional interventions continue to be used to improve child growth, but they have not been as effective as expected, suggesting there are other factors contributing to poor growth, such as EED.[7,8] It has been hypothesized that poor sanitation leading to recurrent exposure to fecal pathogens might be a potential cause of EED.[9] In order to mount a proper defense against these repeated infections, the immune system requires a wide variety of nutrients.[10] A cascade of pro-

inflammatory cytokines has also been proposed as a potential mechanism for the development of EED[11], as has mitochondrial dysfunction[12] and protein expression related to the innate immune system.[9]

A study in Bangladesh found higher levels of pro-inflammatory immune markers to be associated with lower neurodevelopmental scores.[11] This study corroborates a previous study in Tanzania which reported associations linking EED biomarkers to poor child development potentially through a related immune activation pathway with components previously implicated in neuroplasticity and neurogenesis.[2] Current interventions to address EED include the use of anti-inflammatory drugs, antimicrobials, nutritional supplements, and improved water, sanitation, and hygiene (WASH).[13]

The WASH Benefits trials were a pair of 2-year cluster randomized controlled trials examining nutrition, water, sanitation and handwashing interventions in rural Bangladesh and Kenya.[7,8] The Bangladesh trial reported reductions in enteric viral and parasitic infections[14–16], early reductions in intestinal permeability and inflammation[15], and improvements in linear growth[7] and child development[17] in the intervention arms. In Kenya, there were decreased *Ascaris* infections[18] and improved child motor function in the first year without any improvements in the second[19]. Considering these findings, this observational study aims to assess the relationship between markers of environmental enteric dysfunction (EED) and subsequent child development among WASH Benefits participants in Kenya and Bangladesh.

2. Study hypotheses, exposures and outcomes

Exposures:

Details describing the primary EED substudy have been previously published, with the following exposures previously assessed as outcomes.[15] The exposures included in this study are six measures for environmental enteric dysfunction: fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and regenerating gene 1β (REG1B) and urinary lactulose and mannitol (also summarized in the table below). Each of these biomarkers are associated with various presentations of EED: intestinal permeability (alpha-1-antitrypsin, lactulose and mannitol); inflammation (myeloperoxidase, neopterin); and intestinal repair (REG1B).

EED Mechanism	Biomarker	Source
Permeability	Lactulose	Urine
	Mannitol	
	Alpha-1-antitrypsin (A1AT)	Stool
Inflammation	Myeloperoxidase (MPO)	
	Neopterin (NEO)	
Repair	Regenerating Gene 1β (REG1B)	

To reduce inter-laboratory variation, feces samples from Kenya and Bangladesh were assayed in the same International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) laboratory. Study staff followed enzyme-linked immunosorbent assay kit protocols for fecal

alpha-1-antitrypsin, myeloperoxidase, neopterin, and REG1B. Before being administered a lactulose and mannitol solution (250 mg/mL of lactulose and 50 mg/mL of mannitol dosed at 2 mL/kg to each child up to 20 mL), a single urine sample was collected from each child. For the 5 hours following the LM solution administration, all urine was collected and pooled together. The latter sample was analyzed for lactulose and mannitol recovery using high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry at Wagner College (Staten Island, New York). In Bangladesh, all biomarkers were assessed at ages 3, 14, and 28 months, except REG1B, which was only measured at 14 months. In Kenya, all biomarkers were assessed at ages 6, 17, and 22 months, except REG1B, which was not measured. The EED biomarkers will be individually assessed and in exploratory analyses, we will create a composite score of the biomarkers using principal component analysis (PCA).[20]

This study will utilize biomarker measurements from children in all arms of the EED substudy: the control, nutrition, combined WSH, and combined nutrition plus WSH (N+WSH) arms. Treatment arm assignment will be tested as a potential confounder (see Covariate Pre-screening and Selection section for details below).

Outcomes:

Different child development tests were administered when the child was 1 and 2 years old.

At age 1 year, in Kenya and Bangladesh, staff conducted an at-home assessment, supplemented by a parental report, of the attainment of the World Health Organization's (WHO) six motor development milestones: sitting without support, hands-and-knees crawling, standing with assistance, walking with assistance, standing alone, and walking alone.[17,19]

At age 1 and 2 years, the study team in Bangladesh administered the MacArthur-Bates Communicative Development Inventories,[17] which has been culturally adapted and validated for use in Bangladesh.[21] The MacArthur-Bates Communicative Development Inventories measure language development (i.e., the understanding and speaking of words) using a parental report of the child's language development. The MacArthur-Bates Communicative Development Inventories was not assessed in Kenya.

When the child was 2 years old, parents were asked about their child's development using the Extended Ages and Stages Questionnaire (EASQ)--in both Kenya and Bangladesh--and the children were asked to demonstrate specific behaviors.[17,19] The EASQ consists of three domains: communication, gross motor, and personal-social.[17,19] The EASQ has previously been successfully employed in low-resource settings; direct assessment was added to 25% of the items when it was adapted and validated for use in LMICs.[17,19,23] The outcomes for EASQ at 2 years old correspond to each of the three domains (communication, gross motor, and personal-social) and a combined global EASQ score.

For the EASQ and Communicative Development Inventories, we will create reference distributions for each 2-month age band by Z-scoring outcome scores from the control group.

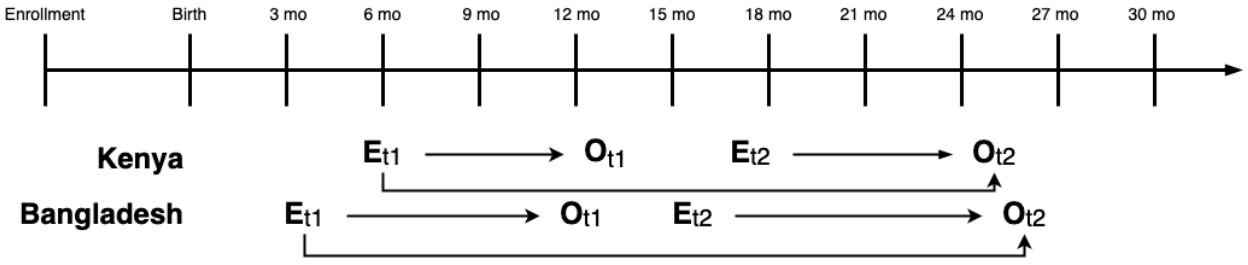
Age-standardized Z-scores for the rest of the sample will be generated by standardizing scores to the reference distribution for each age band.

Study hypotheses:
See Appendix 1 for specific hypotheses.

Exposure timing of EED biomarkers: Follow-up 1 (t_1) measurements occurred when children were at mean age of 3 months in Bangladesh and 6 months in Kenya. Follow-up 2 (t_2) is defined as the measurement when the mean child age was 14 months in Bangladesh and 17 months in Kenya. Follow-up 3 (t_3) is defined as the measurement when the mean child age was 28 months in Bangladesh and 22 months in Kenya.

Outcome timing of child development measures: Follow-up 2 (Year 1) measurements occurred when the children were at mean age of 11 months in Bangladesh and 12 months in Kenya. Follow-up 3 (Year 2) measurements occurred when the children were at mean age 26 months in Bangladesh and 25 months in Kenya.

Study Measurement Timeline



	IQR			
	Year 1		Year 2	
	E_{t1}	O_{t1}	E_{t2}	O_{t2}
Kenya	4 - 6 mo	11 - 13 mo	15 - 18 mo	23 - 26 mo
Bangladesh	1 - 4 mo	10 - 13 mo	13 - 15 mo	24 - 27 mo

Legend
E: Exposure (EED Markers)
O: Outcome (Child Development)

* To simplify analyses the exposures measured at the Follow-Up 3 were considered "concurrent" and were not assessed.

As outlined below, the EED biomarkers will be assessed individually, and in exploratory analyses, we will create a composite score of the biomarkers using principal component analysis.

Bangladesh:

1. Fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and urinary lactulose and mannitol levels measured at Follow-up 1 (month 3) are negatively associated with child development measured at Follow-up 2 (Year 1).

   Exposure: alpha-1-antitrypsin, myeloperoxidase, neopterin, lactulose, and mannitol levels at Follow-up 1 (month 3)

Outcome: child development at Follow-up 2 (Year 1; MacArthur-Bates Communicative Development Inventories Z scores, gross motor milestone achievement assessed with the WHO module, sum scores of the 2nd, 4th, 5th, and 6th WHO gross motor milestones)

2. Fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and urinary lactulose and mannitol levels measured at Follow-up 1 (month 3) and Follow-up 2 (month 14) are negatively associated with child development measured at Follow-up 3 (Year 2).

   Exposure: alpha-1-antitrypsin, myeloperoxidase, neopterin, lactulose, and mannitol levels at Follow-up 1 (month 3) and Follow-up 2 (month 14)
   Outcome: child development at Follow-up 3 (Year 2; communication, gross motor, personal social, and combined Z scores as measured by the EASQ, MacArthur-Bates Communicative Development Inventories Z scores)

3. Fecal REG1B levels measured at Follow-up 2 (month 14) are negatively associated with child development measured at Follow-up 3 (Year 2).

   Exposure: REG1B at Follow-up 2 (month 14)
   Outcome: child development at Follow-up 3 (Year 2; communication, gross motor, personal social, and combined Z scores as measured by the EASQ, MacArthur-Bates Communicative Development Inventories Z scores)

<u>Kenya:</u>

4. Fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and urinary lactulose and mannitol levels measured at Follow-up 1 (month 6) are negatively associated with child development measured at Follow-up 2 (Year 1).

   Exposure: alpha-1-antitrypsin, myeloperoxidase, neopterin, mannitol, and lactulose at Follow-up 1 (month 6)
   Outcome: child development at Follow-up 2 (Year 1; gross motor milestone achievement assessed with the WHO module and sum scores of the 2nd, 4th, 5th, and 6th WHO gross motor milestones)

5. Fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and urinary lactulose and mannitol levels at Follow-up 1 (month 6) and Follow-up 2 (month 17) are negatively associated with child development measured at Follow-up 3 (Year 2).

   Exposure: alpha-1-antitrypsin, myeloperoxidase, neopterin, mannitol, and lactulose at Follow-up 1 (month 6) and Follow-up 2 (month 17)
   Outcome: child development at Follow-up 3 (Year 2; communication, gross motor, personal social, and combined Z scores as measured by the EASQ)

3. Analysis

Nested observational analyses

We will perform a unique analysis for each exposure-outcome association (e.g., alpha-1-antitrypsin at age 3 months and child development at Year 2), recognizing the analyses could be complicated by non-linear or other complex exposure-outcome relationships. The following approach will be customized to suit each individual analysis.

First, we will summarize relationships between EED biomarkers and child development by performing visual exploratory analyses then modeling using cubic splines, leveraging generalized cross-validation to calculate bandwidth.[24] We plan to estimate Bayesian 95% simultaneous confidence intervals around our fitted curves.[25]

To account for the possibility of a nonlinear relationship, we will use natural smoothing splines (a generalized additive model)—adjusting for confounding variables--to summarize mean age-standardized Communicative Development Inventories Z scores (comprehension score, expressive language score); WHO gross motor milestone scores; the sum scores of the 2nd, 4th, 5th, and 6th WHO gross milestone scores; and age-standardized EASQ Z scores (communication Z score, gross motor Z score, personal social Z score, combined Z-score) across EED marker distributions, unadjusted and adjusted for potential confounders. We will then plot the generalized additive model curves between exposures and outcomes, along with the simultaneous confidence intervals. We will additionally report the estimated differences and confidence intervals for the 25th and 75th percentiles of each exposure distribution. Due to the fact that some children skipped some WHO motor milestones (e.g., completed milestone 4 without first completing milestone 3), we will estimate associations between EED markers and age of attainment for each WHO motor milestone by calculating hazard ratios using a complementary log-log link and baseline hazard fit with monotonic cubic splines.[17]

We will additionally report estimated differences, relative risks, or hazard ratios (along with 95% confidence intervals) between the 25th and 75th percentile of each exposure distribution. We will report both raw p-values and p-values adjusted for multiple testing (by controlling the false discovery rate) within each hypothesis using the Benjamini-Hochberg procedure. We will also report E-values for the point estimates and lower bounds of the 95% confidence intervals to provide an estimate around the strength of unmeasured confounding needed to explain associations if the null hypothesis were true.[26]

Covariate pre-screening and selection

All analyses will control for child age, sex, and other covariates found to be significantly related to the outcome in bivariate analyses. Based on literature review, subject area knowledge (e.g., previous analyses), we have selected covariates that may be associated with the exposures and the outcomes but are not likely intermediate variables on the causal pathway between exposure and outcome or shared common effects of both exposure and outcome (see Figure 1 and full list of covariates below). Thus, we do not expect there to be collinearity between covariates. We will statistically assess confounding for each covariate meeting these criteria by using the likelihood ratio test to assess the association between each outcome and each

covariate and will include covariates with a p-value<0.20 in the analysis. We will also exclude covariates that have little variation in the study population (e.g., prevalence <5%). Effect modification and covariate interaction will be assessed within each model, described later.

In accordance with the main study's analysis plan (https://osf.io/63mna/), we will consider and test for a set of potential confounding variables for Kenya and Bangladesh independently in our adjusted models (see below).

Enrollment characteristics:

- Child sex
- Child birth order (first born, second born or greater)
- Mother's age (years)
- Mother's height (cm)
- Mother's education level (no education, primary, secondary)
    - Bangladesh: no education, primary, secondary
    - Kenya: none or incomplete primary, complete primary, any secondary
- Household food insecurity (4-level HFIAS categories) (Bangladesh only)
- Household Hunger Scale (3-level HHS) (Kenya only)
- Number of children < 18 years in the household
- Number of individuals living in the compound
- Distance (in minutes) to the household's primary drinking water source
- Housing materials (each material tested separately)
    - Bangladesh: floor, walls, roof
    - Kenya: floor, roof
- Asset-based household-wealth variable (continuous), calculated from the first principal component of a principal components analysis of the following household assets:
    - Bangladesh: electricity, wardrobe, table, chair or bench, watch or clock, khat, chouki, working radio, working black/white or color television, refrigerator, bicycle (not child's toy), motorcycle, sewing machine, mobile phone, land phone, number of cows, number of goats, number of chickens.
    - Kenya: electricity, clock, working radio, working black/white or color television, bicycle (not child's toy), motorcycle, sewing machine, mobile phone, land phone, stove, number of cows, number of goats, number of dogs, number of poultry.

We will assess the following characteristics, measured at follow-up, for confounding (see the directed acyclic graph in Figure 1 below).

- Treatment arm (control, nutrition, WSH, N+WSH). EED biomarkers were collected in the active control arm in Kenya and the passive control arm in Bangladesh.
- Child's length-/height-for-age (LAZ/HAZ) and weight-for-age (WAZ) will be tested as a potential confounder for subsequent time points
- Month of measurement will be tested as a potential confounder for concurrent and subsequent time points
- Child age (in days) will be tested as a potential confounder for concurrent and subsequent outcomes

- The Maternal Perceived Stress Scale (PSS) score at Follow-up 3 will be assessed for confounding of Follow-up 3 outcomes in Kenya and Bangladesh.
- The WHO Health and Life Experiences Survey, which measured maternal lifetime cumulative exposure to intimate partner violence will be assessed for confounding of outcomes in Bangladesh only (this survey was not administered in Kenya). Intimate partner violence contributes to maternal depression and has been linked to increased parenting stress and to harsher maternal parenting practices.[28]
- Maternal depressive symptoms:
    Bangladesh: Center for Epidemiologic Studies Depression (CES-D) Scale score [31] measured at Follow-up 2 will be assessed for confounding of Follow-up 2 and 3 outcomes. The CES-D score measured at Follow-up 3 will be assessed for confounding of Follow-up 3 outcomes. The CES-D score will be a continuous measure, as no cutoff to identify depression has been validated in Bangladesh. A study in Vietnam found maternal depressive symptoms' effect on child socioemotional development to be mediated by parental efficiency and early parenting practices.[29,30] Furthermore, inflammation, hormones, and neuroendocrine processes have all been associated with postpartum depression.[18] Intimate partner violence and life stress can also impact maternal depression.[31]
    - Kenya: Patient Health Questionnaire (PHQ) score measured at Follow-up 2 will be assessed for confounding of Follow-up 2 and 3 outcomes, and the Follow-up 3 PHQ score will be assessed for confounding of Follow-up 3 outcomes.

<u>Effect modification analyses:</u>

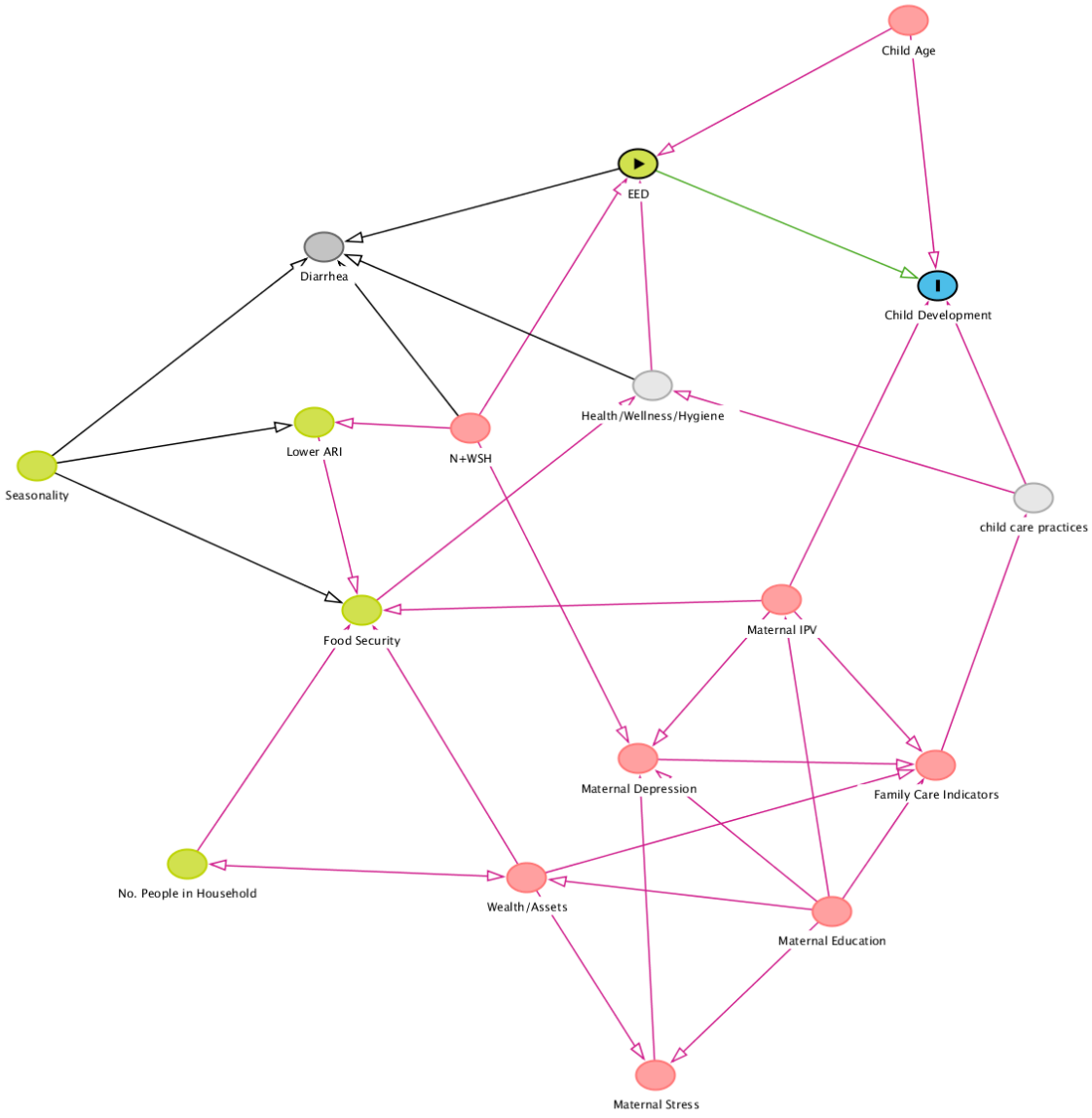
We will assess possible modification of the associations between EED biomarkers and child development by the following variables as previously described (https://osf.io/szd4g/):

- Parental perceived stress (Bangladesh, Follow-up 3)
- Maternal perceived stress (Kenya, Follow-up 3)
- Maternal depressive symptoms (Bangladesh and Kenya, Follow-up 2 and Follow-up 3)
- Maternal cortisol (Bangladesh only, measured during pregnancy)
- Child stress (pre-stressor cortisol, post-stressor cortisol, cortisol reactivity, and F2-isoprostanes) (Bangladesh only, Follow-up 3)
- Cumulative maternal exposure to intimate partner violence at Follow-up 2 and Follow-up 3 (Bangladesh only)
- Stimulation in the home as measured by the stimulation subscale (number of stimulation activities the mother, father, or any other caregiver over age 15 years has completed with the child in the past 3 days) in the Family Care Indicators (FCI) questionnaire[21] administered at Follow-up 2 and Follow-up 3 in Bangladesh and Kenya. It will be defined as the total number of activities an adult participated in, with a maximum score of 6.

We will assess effect modification by including interaction terms between the proposed modifiers and each EED marker within generalized additive models (GAMs). We will compare

GAMs with interaction terms to GAMs with only main effects for the EED marker and the modifier variable using likelihood-ratio tests. We will use the p-value from the likelihood-ratio test to assess the significance of effect modification (p-value<0.2). We will report the predicted difference between the 1st and 3rd quartile of each EED marker when setting the value of the effect modifier variable to the 1st and 3rd quartile of its distribution (for continuous modifiers) or to 0 or 1 (for binary variables) for all children. We consider these effect-modification analyses as pre-specified exploratory analyses.

Figure 1. A conceptual model of potential confounding variables for the Bangladesh and Kenya EED biomarkers and child development studies.



† Maternal exposure to intimate partner violence (IPV) was only measured in Bangladesh.

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Appendix 1: Specific Hypotheses

Bangladesh:

H1. Fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and urinary lactulose and mannitol levels measured at Follow-up 1 (month 3) are negatively associated with child development measured at Follow-up 2 (Year 1).

Fecal alpha-1-antitrypsin measured at Follow-up 1 (month 3)	WHO motor milestones at Follow-up 2 (Year 1)
Fecal alpha-1-antitrypsin measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 2 (Year 1)
Fecal myeloperoxidase measured at Follow-up 1 (month 3)	WHO motor milestones at Follow-up 2 (Year 1)
Fecal myeloperoxidase measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 2 (Year 1)
Fecal neopterin measured at Follow-up 1 (month 3)	WHO motor milestones at Follow-up 2 (Year 1)
Fecal neopterin measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 2 (Year 1)
Urinary lactulose measured at Follow-up 1 (month 3)	WHO motor milestones at Follow-up 2 (Year 1)
Urinary lactulose measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 2 (Year 1)
Urinary mannitol measured at Follow-up 1 (month 3)	WHO motor milestones at Follow-up 2 (Year 1)
Urinary mannitol measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 2 (Year 1)

H2. Fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and urinary lactulose and mannitol levels measured at Follow-up 1 (month 3) and Follow-up 2 (month 14) are negatively associated with child development measured at Follow-up 3 (Year 2).

Fecal alpha-1-antitrypsin measured at Follow-up 1 (month 3)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal alpha-1-antitrypsin measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)
Fecal alpha-1-antitrypsin measured at Follow-up 2 (month 14)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal alpha-1-antitrypsin measured at Follow-up 2 (month 14)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)
Fecal myeloperoxidase measured at Follow-up 1 (month 3)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal myeloperoxidase measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)
Fecal myeloperoxidase measured at Follow-up 2 (month 14)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal myeloperoxidase measured at Follow-up 2 (month 14)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)
Fecal neopterin measured at Follow-up 1 (month 3)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal neopterin measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)
Fecal neopterin measured at Follow-up 2 (month 14)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal neopterin measured at Follow-up 2 (month 14)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)
Urinary lactulose measured at Follow-up 1 (month 3)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)

Urinary lactulose measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)
Urinary lactulose measured at Follow-up 2 (month 14)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Urinary lactulose measured at Follow-up 2 (month 14)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)
Urinary mannitol measured at Follow-up 1 (month 3)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Urinary mannitol measured at Follow-up 1 (month 3)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)
Urinary mannitol measured at Follow-up 2 (month 14)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Urinary mannitol measured at Follow-up 2 (month 14)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)

H3. REG1B levels measured at Follow-up 2 (14 months) are negatively associated with cognitive development measured at Follow-up 3 (Year 2).

REG1B measured at Follow-up 2 (month 14)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
REG1B measured at Follow-up 2 (month 14)	MacArthur-Bates Communicative Development Inventories scores at Follow-up 3 (Year 2)

Kenya:

H4. Fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and urinary lactulose and mannitol levels measured at Follow-up 1 (month 6) are negatively associated with child development measured at Follow-up 2 (Year 1).

Fecal alpha-1-antitrypsin measured at Follow-up 1 (month 6)	WHO motor milestones at Follow-up 2 (Year 1)

Fecal myeloperoxidase measured at Follow-up 1 (month 6)	WHO motor milestones at Follow-up 2 (Year 1)
Fecal neopterin measured at Follow-up 1 (month 6)	WHO motor milestones at Follow-up 2 (Year 1)
Urinary lactulose measured at Follow-up 1 (month 6)	WHO motor milestones at Follow-up 2 (Year 1)
Urinary mannitol measured at Follow-up 1 (month 6)	WHO motor milestones at Follow-up 2 (Year 1)

H5. Fecal alpha-1-antitrypsin, myeloperoxidase, neopterin, and urinary lactulose and mannitol levels measured at Follow-up 1 (month 6) and Follow-up 2 (month 17) are negatively associated with child development measured at Follow-up 3 (Year 2).

Fecal alpha-1-antitrypsin measured at Follow-up 1 (month 6)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal alpha-1-antitrypsin measured at Follow-up 2 (month 17)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal myeloperoxidase measured at Follow-up 1 (month 6)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal myeloperoxidase measured at Follow-up 2 (month 17)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal neopterin measured at Follow-up 1 (month 6)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Fecal neopterin measured at Follow-up 2 (month 17)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Urinary lactulose measured at Follow-up 1 (month 6)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Urinary lactulose measured at Follow-up 2 (month 17)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)
Urinary mannitol measured at Follow-up 1 (month 6)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)

Urinary mannitol measured at Follow-up 2 (month 17)	Extended Ages and Stages Questionnaire at Follow-up 3 (Year 2)