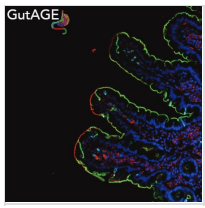

Oct 26, 2022

Participant Registration Form For Mapping the Early Childhood Gut Across Ancestry, Geography, and Environment (Gut-AGE)

Fatima Zulqarnain[1], Stephanie Regis[2], elsy.ngwa[2], Asra Usmani[3], jason.boisvert[2], Arg7Ef[1], Sana Syed[1], Jocelyn Silvester[2], jay.thiagarajah[2]

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HUMAN CELL ATLAS

Fatima Zulqarnain

ABSTRACT

The gastrointestinal (GI) system has a critical role in growth and development during infancy and early childhood, with early development continuing to influence health outcomes into adulthood. Several GI diseases are currently being characterized at single-cell resolution; however, the interpretation of this data is limited by the lack of well-annotated reference data, particularly from healthy infants and young children. The aim of this project is to map the healthy gut in infants and children (age 0-5 years) during a critical developmental window that impacts long-term health outcomes and is shaped by genetics and the environment. We will map early gut development across populations with diverse ancestry and geography, at single-cell resolution, and with linked contextual data on tissue morphology, genetic background, social determinants of health, and environmental exposures. The participant registration form for this study aims to capture clinical metadata – including demographic data (age, weight, height/length, gestational age at birth, etc.), clinical data (reason for biopsy), and nutritional information – to construct a well-annotated reference dataset.

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PROTOCOL INTEGER ID

71010

To be filled out by the study team

1 PARTICIPANT REGISTRATION FORM

The first part of the form collects registration information from each participant. The purpose of this section is to ensure that the study team has all relevant participant information for accessing participant health records at their site of care. This information will allow study members to link their protected health information (PHI) to their study ID and confirm that participants meet inclusion and/or exclusion criteria at the time of enrollment. Information about the physician involved in the study procedures will help track any protocol deviations or adverse events. This section will also be used to document study consent procedures.

At the end of the consent section, there is a free text space for any comments from the registration process, which can be used to document the refusal of consent for certain samples, the need for accommodations such as an interpreter, etc.

MMM: months (3 letter - Jan, Feb, Mar...)

A	B	C
PARTICIPANT REGISTRATION FOR (ADD STUDY NAME HERE)		
Participant Name	(First Name)	(Last Name)
Participant Study Registration Number:		
Study enrollment date (DD-MMM-YYYY):		
Local Medical Record Number:		
Birthdate (DD-MMM-YYYY):		
Sex assigned at birth:	☐ Male	☐ Female
Name of Site PI:		
Name of Research Staff:		
Name of Physician Performing Endoscopy:		
Inclusion Criteria		
If the response to any of the following questions is “No” it is likely that the subject is NOT eligible for participation in this study, please discuss with the site-PI.		
Is the participant less than 6 years old?	☐ Yes	☐ No
Will the participant undergo an upper or lower GI endoscopy?	☐ Yes	☐ No
Is it possible to obtain appropriate consent? "(i.e., child is >10 kg or both parents available for consent) "	☐ Yes	☐ No
Exclusion Criteria		
If the response to any of the following questions is “Yes” it is likely that the subject is NOT eligible for participation in this study, please discuss with the site-PI.	☐ Yes	☐ No
Does the participant have known coagulopathy, thrombocytopenia (<150K platelets), or a bleeding disorder?	☐ Yes	☐ No
Does the participant have a known connective tissue disorder	☐ Yes	☐ No
Are there any other conditions that would make the participant ineligible for this study?	☐ Yes	☐ No
If yes, please describe briefly:		
Study Consent Procedures		
Was consent obtained?	☐ Yes	☐ No
Date consent obtained (DD-MMM-YYYY):		
Who signed the consent?	☐ Subject	☐ Legal Guardian
	☐ Mother	☐ Other: _____
	☐ Father	
Initials of Research Staff who obtained consent:		
Comments from registration process:		

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2 PARTICIPANT'S CLINICAL HISTORY

The clinical history section is taken from the participant's health record. This section can be tailored to the organ system that is the focus of the study. The categories in this protocol will be used to construct a well-annotated, gastrointestinal-focused reference dataset. It was built with input from three American Academy of Pediatrics board-certified pediatric gastroenterologists; thus variables to be collected are focused on the gastrointestinal system.

Report all variables to two decimal places (e.g., ###.##) when possible.

A	B	C	D
PARTICIPANT'S CLINICAL HISTORY			
Reason for endoscopy:	☐ Foreign Body Removal	☐ Diarrhea	☐ Failure to thrive
	☐ Dysphagia	☐ Constipation	☐ Abdominal Pain
	☐ Reflux	☐ Nausea/vomiting	☐ Routine Clinical Care
	☐ GI bleeding "(e.g., hematemesis, melena)"	☐ Aspiration	☐ Other: _____
Anthropometrics			
Current height or length (cm)		Date measured:	
Current weight (kg)		Date measured:	
Are growth records available prior to study?	☐ Yes	☐ No	
Laboratory			
Complete Blood Count (CBC)	☐ Yes	☐ No	(if data is available)
If yes, date of CBC (DD-MMM-YYYY):			
WBC (K cells/μL)			
Hemoglobin (g/dL)			
Hematocrit (%)			
Platelets (K cells/μL)			
Neutrophils (K cells/μL)			
Lymphocytes (K cells/μL)			
Eosinophils (K cells/μL)			
Liver and Kidney Panel	☐ Yes	☐ No	(if data is available)
If yes, date of labs (DD-MMM-YYYY):			
Total Bilirubin (mg/dL)			
Direct Bilirubin (mg/dL)			

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Albumin (g/dL)			
Alkaline Phosphate (U/L)			
Aspartate transaminase (U/L)			
Alanine transaminase (U/L)			
Blood urea nitrogen (mg/dL)			
Creatinine (mg/dL)			
Other labs			
Erythrocyte sedimentaion rate (mm/hr)		Date measured:	
C-reactive protein (mg/dL)		Date measured:	
Iron, plasma (mcg/dL)		Date measured:	
Total iron binding capacity (mcg/dL)		Date measured:	
Urea (mm/L)		Date measured:	
Urine sodium (mEq/L)		Date measured:	
Gamma-glutamyl Transferase (U/L)		Date measured:	
Ferritin (ng/mL)		Date measured:	
Stool Studies			
CliniTest-reducing substance (during feeding)	☐ Positive	☐ Negative	Date measured:
Fecal Elastase (µg/g)		Date measured:	
Alpha1-Antitrypsin (mg/g)		Date measured:	
Stool pH (during feeding)		Date measured:	
Calprotectin (µg/mg)		Date measured:	
Lactoferrin (µg/ml)		Date measured:	
Stool Electrolytes	☐ Yes	☐ No	(if data is available)
If yes, date of labs (DD-MMM-YYYY):			
Sodium (mmol/L)			
Potassium (mmol/L)			
Chlorine (mmol/L)			
Bicarbonate (mEq/L)			
Vitamin Deficiency			
Vitamin A	☐ Yes	☐ No	☐ Unknown
If yes, describe - provide lab value & range if present			
Vitamin D	☐ Yes	☐ No	☐ Unknown
If yes, describe - provide lab value & range if present			
Vitamin E	☐ Yes	☐ No	☐ Unknown
If yes, describe - provide lab value & range if present			
Vitamin K	☐ Yes	☐ No	☐ Unknown

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If yes, describe - provide lab value & range if present			
Gastroenterology Procedural History	☐ Yes	☐ No	☐ Unknown
If yes, specify	☐ Yes	☐ No	Date measured:
If abnormal, select all locations that apply	☐ Esophagus		
	☐ Stomach		
	☐ Duodenum		
	☐ Colon		
	☐ Ileum		

To be filled out by the patient's family

3 The following questionnaire can be given to the family to fill out, or be filled out in conjunction with one of the study staff, or on the telephone at a later date.

CHILD'S DEMOGRAPHICS

Demographic data on the race and/or ethnicity of patients is captured to ensure that the study population is representative of the whole population so that any conclusions drawn from the research are generalizable to larger groups. In 1993, the National Institutes of Science (NIH), published the Revitalization Act, which states "since a primary aim of the research is to provide scientific evidence leading to a change in health policy or standard of care, it is imperative to determine whether the intervention or therapy being studied affects women or men or members of minority groups and their subpopulations differently."

In 1997, the NIH issued revisions to the Standards for the Classification of Federal Data on Race and Ethnicity. These standards are commonly used for federal data collection purposes, such as the census, as well as clinical research. The revised standards contain five **minimum** categories for race: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, and White. There are two categories for ethnicity: "Hispanic or Latino" and "Not Hispanic or Latino." The categories and definitions provide a common language to promote uniformity and comparability of data on race and ethnicity. In the questionnaire below, and in subsequent questionnaires about the race of other members of the patient's family, we have collapsed race and ethnicity into one question with the possibility to check more than one box.

As the NIH recommends the above five as minimum criteria, after an extensive literature review, we expanded the "Asian" category to include the East, Southeast, and South Asian to allow for more comprehensive collection of racial data from Asia. The Canadian Census categories (Visible Minority and Population Group Reference Guide, Census of Population, 2021 (statcan.gc.ca)) were used for this expansion.

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A	B
Select all identities that apply, if there are additional identities not described below, please self-describe using Other.	
Race and/or Ethnicity:(Select all that apply)	☐ Native American/Alaska Native
	☐ Black or African-American
	☐ Middle Eastern/North African
	☐ East Asian (e.g. Chinese, Japanese, Korean)
	☐ Southeast Asian (e.g. Vietnamese, Filipino)
	☐ South Asian (e.g. Indian, Pakistani)
	☐ White
	☐ Native Hawaiian or Other Pacific Islander
	☐ Hispanic or Latino
	☐ Not aligned with above categories: _____ (e.g., Ashkenazi Jewish, French Canadian, Afro-Caribbean, etc.)

4 CHILD'S BIRTH HISTORY

This project is also investigating geography and ancestry. Patient geographical data at the time of their birth is used can be used to study ancestry, environmental exposures, and migration.

A child's birth history also gives pertinent information about their development. Premature birth has been implicated in an increased risk of intraventricular hemorrhage, necrotizing enterocolitis (NEC), and retinopathy of prematurity, all of which can lead to increased morbidity in childhood (Ward et al).

Small for gestational age (SGA) is defined as a birth weight of less than the 10th percentile for gestational age, according to the 2021 American College of Obstetrics and Gynecology Bulletin. Infants who are SGA have traditionally been divided into two groups: (1) constitutionally normal infants who are SGA and (2) infants who are SGA because of growth restriction. Infants who are constitutionally normal will have a birth weight less than the 10th percentile on population-adjusted growth curves due to maternal height, weight, and/or ethnicity, and will have a decreased risk of perinatal morbidity and mortality compared to their growth-restricted counterparts (Carberry et al). The burden of fetal growth-restricted SGA is higher in resource-poor countries. These children are at higher risk of prematurity, neonatal asphyxia, hypothermia, hypoglycemia, hypocalcemia, polycythemia, sepsis, and death (Liu et al).

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A	B	C
CHILD'S BIRTH HISTORY		
Country of birth?		
Estimated date of arrival in present country? (DD-MMM-YYYY):		☐ Unknown or Does not apply
Gestational age (weeks):		☐ Unknown
Expected due date if gestational age not remembered		☐ Unknown
Birth weight (g)		☐ Unknown
Birth length (cm)		☐ Unknown
Mode of delivery	☐ Vaginal ☐ C section	☐ Unknown

Ward RM, Beachy JC (2003). Neonatal complications following preterm birth. BJOG. http://10.1016/s1470-0328(03)00012-0

Liu J, Wang XF, Wang Y, Wang HW, Liu Y (2014). The incidence rate, high-risk factors, and short- and long-term adverse outcomes of fetal growth restriction: a report from Mainland China. Medicine. http://10.1097/MD.0000000000000210

ACOG (2021). Fetal Growth Restriction: ACOG Practice Bulletin, Number 227. Obstet Gynecol. http://10.1097/AOG.0000000000004251

Carberry AE, Gordon A, Bond DM, Hyett J, Raynes-Greenow CH, Jeffery HE (2014). Customised versus population-based growth charts as a screening tool for detecting small for gestational age infants in low-risk pregnant women. Cochrane Database Syst Rev. http://CD008549

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5 CHILD'S PAST MEDICAL HISTORY

A complete medical history includes an in-depth inquiry into the patient's past medical issues, which includes all diseases or illnesses currently being treated, as well as any other issues that may have residual effects on the patient's health. As this project focuses on the gastrointestinal system, discussions with three board-certified pediatric gastroenterologists have led us to include the history of common, uncommon, and systemic conditions that may have gastrointestinal manifestations (such as allergies) in this section.

Heavy metals are high atomic weight elements that naturally occur in the environment and have a density at least 5-times greater than that of water. These include arsenic, cadmium, chromium, copper, lead, mercury, and nickel (Tchounwou et al). Heavy metals are used in a variety of industrial processes and can cause acute and/or chronic toxicity due to environmental exposures. These metals may cause gastrointestinal, kidney, and nervous system dysfunction, vascular damage, immune dysregulation, birth defects, and cancer, among other pathologies (Balali-Mood et al). The table below summarizes common heavy metals, their sources of exposure, and the acute and chronic effects of toxicity.

Heavy metal	Source of exposure	Effects of Toxicity	
		Acute	Chronic
Arsenic	wood preservatives, pesticides, and fungicides	Nausea, vomiting, diarrhea Painful neuropathy Encephalopathy	Diabetes, skin pigmentation, polyneuritis
Chromium	Steel industries, tanneries	Acute renal failure Gastrointestinal hemorrhage	Lung cancer, pulmonary fibrosis
Copper	Pesticides, fertilizers, smelting	Gastrointestinal hemorrhage, multiorgan dysfunction	Wilson's disease: psychiatric symptoms, liver dysfunction, arthritis
Mercury	Medical waste, coal combustion	Fever, vomiting, diarrhea, caustic gastroenteritis	Neurological damage: ataxia, muscle weakness, numb limbs, disturbance in speech
Lead	Herbicides, battery waste, fuel, insecticides, wall paint	Vomiting, diarrhea, abdominal pain	Osteoporosis, neurologic damage, anemia, developmental delay and failure to thrive in children

Others: Nickel, Cadmium

adapted from Balali-Mood et al, and Verma et al.

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A	B	C	D
CHILD'S PAST MEDICAL HISTORY			
Prior hospitalizations	☐ Yes	☐ No	☐ Unknown
Prior surgical therapies (e.g. tonsillectomy, appendectomy)	☐ Yes	☐ No	☐ Unknown
Prior lead exposure testing	☐ Yes	☐ No	☐ Unknown
If yes, what were the results?	☐ Normal	☐ Abnormal	☐ Unknown
Failure to thrive as defined by physician	☐ Yes	☐ No	☐ Unknown
Gastrointestinal Conditions			
Inflammatory Bowel Disease (Crohn's Disease or Ulcerative Colitis)	☐ Yes	☐ No	☐ Unknown
Irritable Bowel Syndrome	☐ Yes	☐ No	☐ Unknown
Celiac disease	☐ Yes	☐ No	☐ Unknown
EoE (Eosinophilic Esophagitis)	☐ Yes	☐ No	☐ Unknown
H. pylori Gastric Infection	☐ Yes	☐ No	☐ Unknown
Allergic enteritis/milk intolerance	☐ Yes	☐ No	☐ Unknown
Other Gastrointestinal Disease:	☐ Yes	☐ No	☐ Unknown
If yes, list:			
Developmental delay	☐ Yes	☐ No	☐ Unknown
Asthma	☐ Yes	☐ No	☐ Unknown
Eczema	☐ Yes	☐ No	☐ Unknown
Food allergies	☐ Yes	☐ No	☐ Unknown
Medication allergies	☐ Yes	☐ No	☐ Unknown
Environmental allergies	☐ Yes	☐ No	☐ Unknown
Other	☐ Yes	☐ No	☐ Unknown
If yes, list:			

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012). Heavy metal toxicity and the environment. Exp Suppl. http://10.1007/978-3-7643-8340-4_6

Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic.. Frontiers in pharmacology. https://doi.org/10.3389/fphar.2021.643972

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Verma N, Sharma R (2017). Bioremediation of Toxic Heavy Metals: A Patent Review.. Recent patents on biotechnology.
https://doi.org/10.2174/1872208311666170111111631

6 CHILD'S MEDICATIONS

Medication history provides information on exposure when trying to identify associations between medications and outcomes. The outcome measured can be related to structural changes in the target tissue or functional changes in the history of the illness.

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A	B	C	D
CHILD'S MEDICATIONS			
Antacids	☐ Yes	☐ No	
If yes, select all that apply:	☐ PPI (e.g., Omeprazole - Zegerid, Pantoprazole - Protonix)		
	☐ H2 Blocker (e.g., Famotidine - Pepcid, Ranitidine - Zantac)		
Probiotics	☐ Yes	☐ No	
Vitamin supplementation	☐ Yes	☐ No	
If yes, select all that apply:	☐ Vitamin D		
	☐ Vitamin K		
	☐ Vitamin A		
	☐ Vitamin B12		
	☐ Vitamin E		
	☐ Other		
Mineral or micronutrient supplementation	☐ Yes	☐ No	
If yes, select all that apply:	☐ Calcium		
	☐ Iron		
	☐ Zinc		
	☐ Other		
Immunosuppressants or Immunomodulators			
If yes, select all that apply:	☐ Oral Steroids		
	☐ IV Steroids		
	☐ Asthma Inhaler		
	☐ Tacrolimus/Sirolimus		
	☐ 6MP		
	☐ AZA		
	☐ Other		
Over-the-counter medications	☐ Yes	☐ No	
Other medications	☐ Yes	☐ No	
If yes, list:			

7 DIETARY QUESTIONNAIRE - 24 HOUR RECALL

The diet questionnaire is based on a publication by the World Health Organization called *Indicators for assessing infant and young child feeding practices (IYCF)* for ages 2 or younger (World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), 2021); it provides 17 IYCF indicators of diet quality. The WHO IYCF indicators and questionnaire were

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selected because they represent validated and comprehensive measures of diet quality. For example, the indicators were used by the Demographic and Healthy Surveys (DHS) and UNICEF Multiple Indicators Cluster Surveys (MICS).

The questions are grouped by nutrient class/type providing a straightforward method of evaluating nutrient status (e.g. Vitamin A, iron, etc) and risk for chronic, noncommunicable diseases. Diet diversity is associated with increased linear growth in children and decreased risk of micronutrient deficiency (World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), 2021). The objective of the diet questionnaire is to understand the participant's approximate macro/micronutrient intake within the past 24 hours which could, in turn, affect what we see in the multiplexed-immunofluorescent images, single-cell sequencing, and other data that we are producing from biopsies and blood collected from participants.

A	B	C	D
DIETARY QUESTIONNAIRE			
Questions about Liquids			
Now I would like to ask you about liquids that [NAME] had yesterday during the day or at night. Please tell me about all drinks, whether [NAME] had them at home, or somewhere else. Yesterday during the day or at ight, did [NAME] have...?			
Plain water?	☐ Yes	☐ No	☐ I don't know
Infant formula, such as [insert local names of common formula]? In the United States, for example: Similac, Enfamil, Neocate, Elecare, Happy Baby, Earth's Best, or Gerber	☐ Yes	☐ No	☐ I don't know
If yes, how many times?			
Milk from animals, including fresh, tinned or powdered?	☐ Yes	☐ No	☐ I don't know
If yes, how many times?			
If yes, was the milk or were any of the milk drinks a sweet or flavored type of milk?	☐ Yes	☐ No	☐ I don't know
Yogurt drinks such as [insert local names of common types of yogurt drinks]?	☐ Yes	☐ No	☐ I don't know
If yes, how many times?			
If yes, was the yogurt or were any of the yogurt drinks a sweetened or flavored type of yogurt drink, such as Danimals or similar liquid yogurt drinks, kefir, or buttermilk ?	☐ Yes	☐ No	☐ I don't know
Chocolate-flavored drinks including those made from syrups or powders?	☐ Yes	☐ No	☐ I don't know

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Fruit juice or fruit-flavored drinks including those made from syrups or powders?	☐ Yes	☐ No	☐ I don't know
Sodas, malt drinks, sports drinks or energy drinks?	☐ Yes	☐ No	☐ I don't know
Tea, coffee, or herbal drinks?	☐ Yes	☐ No	☐ I don't know
If yes, were any of these drinks sweetened or flavored?	☐ Yes	☐ No	☐ I don't know
Clear broth or clear soup?	☐ Yes	☐ No	☐ I don't know
Any other liquids?	☐ Yes	☐ No	☐ I don't know
If yes, what was/were the liquids?			
If yes, was the drink or were any of these drinks sweetened or flavored?	☐ Yes	☐ No	☐ I don't know
When did the participant last eat?	☐ 0-3 hours ago ☐ 3-6 hours ago ☐ 6-12 hours	☐ 12-18 hours ago ☐ 18-24 hours ago	☐ I don't know
Questions about Foods			
Now I would like to ask you about the foods that [NAME] ate yesterday during the day or at night. I will ask you about different. types of foods, and I would like to know whether your child ate the food even if it was combined with foods in a mixed dish like [list common local. examples of mixed dishes]. Please do not answer "yes" for any food or ingredient used in a small amount to add flavo to a dish. Yesterday during the day or at night, did [NAME] eat:			
Yogurt, other than yogurt drinks? (i.e. yogurt eaten with spoon)	☐ Yes	☐ No	☐ I don't know
If yes, how many times?			
Porridge, bread, rice, noodles, pasta, or [insert other commonly consumed grains from including foods made from grains like rice dishes, noodle dishes, etc.]?	☐ Yes	☐ No	☐ I don't know
Pumpkin, carrots, sweet red peppers, squash, or sweet potatoes that are yellow or orange inside? [any additions to this list should meet criteria for defining foods and liquids as 'sources' of vitamin A]	☐ Yes	☐ No	☐ I don't know
Plantains, white potatoes, white yams, manioc, cassava or other starchy vegetables like jicama, parsnips, taro root, turnips, breadfruit, etc. [insert other commonly consumed strachy tubers or starchy tuberous roots that are white or pale inside]?	☐ Yes	☐ No	☐ I don't know

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Dark green leafy vegetables, such as arugula, spinach, broccoli, kale, watercress, lettuce (e.g. romaine) [insert commonly consumed vitamin A-rich dark green leafy vegetables]?	☐ Yes	☐ No	☐ I don't know
Any other vegetables, such as asparagus, eggplant, avocado, beets, cabbage, cauliflower, green pepper, mushroom, lettuce (e.g. iceberg), celery, tomato, etc. [insert commonly consumed vegetables]?	☐ Yes	☐ No	☐ I don't know
Ripe mangoes, ripe papayas, or other vitamin-A rich fruits like cantaloupe, dried peaches, apricot, or ripe passionfruit	☐ Yes	☐ No	☐ I don't know
Any other fruits, such as as apple, banana, blackberry, blueberry, grapefruit, kiwi, orange, honeydew melon, figs, grapes, etc. [insert commonly consumed fruits]?	☐ Yes	☐ No	☐ I don't know
Liver kidney, heart or other organ meats like gizzard, blood sausage, intestines, etc. [insert commonly consumed organ meats]?	☐ Yes	☐ No	☐ I don't know
Sausages, hot dogs, ham, bacon, salami, canned meat, beef jerky/ biltong, or [insert other commonly consumed processed meats]?	☐ Yes	☐ No	☐ I don't know
Any other meat, such asbeef, pork, lamb, goat, chicken, duck, turkey, deer, or [insert other commonly consumed meat]?	☐ Yes	☐ No	☐ I don't know
Eggs?	☐ Yes	☐ No	☐ I don't know
Fresh fish, dried fish or shellfish?	☐ Yes	☐ No	☐ I don't know
Beans, peas, lentils, nuts, seeds, chickpeas or [insert commonly consumed foods made from beans, peas, lentils, nuts, or seeds]?	☐ Yes	☐ No	☐ I don't know
Hard or soft cheese such as cheddar, gouda, American cheese, Swiss cheese, brie, cottage cheese [insert commonly consumed types of cheese]?	☐ Yes	☐ No	☐ I don't know
Sweet foods such as chocolates, candies, pastries, cakes, biscuits, or frozen treats like ice cream and popsicles, or [insert other commonly consumed sentinel sweet foods]?	☐ Yes	☐ No	☐ I don't know
Chips, crisps, puffs, French fries, fried dough, instant noodles, fried plantain snacks or [insert other commonly consumed sentinel fried and salty foods]?	☐ Yes	☐ No	☐ I don't know
Any other solid, semi-solid or soft foods?	☐ Yes	☐ No	☐ I don't know
If yes, list all the solid, semi-solid, or soft foods that do not fit the food groups above:			

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How many times did the participant eat any solid, semi-solid, or soft foods yesterday during the day or night?			

8 FOOD SECURITY

Hunger Vital Sign™ is a 2-question food insecurity screening tool to identify households at risk of food insecurity. he Hunger Vital Sign™ identifies households as being at risk for food insecurity if they answer that either or both of the following two statements is 'often true' or 'sometimes true' (vs. 'never true'). This tool was validated in a survey of 30, 098 families, 23% of which were food insecure and an affirmative response to either question 1 or 2 had a sensitivity of 97% and specificity of 83% and was associated with increased risk of reported poor child health. We added a question regarding food acquisition through federal benefits to assess socioeconomic status.

A	B	C
FOOD SECURITY – U.S HUNGER VITAL SIGN™		
Within the past 12 months we worried whether our food would run out before we got money to buy more	☐ Often true ☐ Sometimes true	☐ Never true ☐ I don't know
Within the past 12 months the food we bought just didn't last and we didn't have money to get more	☐ Often true ☐ Sometimes true	☐ Never true ☐ I don't know
Has the participant's primary or co-primary household ever been eligible for WIC and/or SNAP benefits?	☐ Yes ☐ No	☐ I don't know

Hager ER, Quigg AM, Black MM, Coleman SM, Heeren T, Rose-Jacobs R, Cook JT, Ettinger de Cuba SA, Casey PH, Chilton M, Cutts DB, Meyers AF, Frank DA (2010). Development and validity of a 2-item screen to identify families at risk for food insecurity.. Pediatrics. https://doi.org/10.1542/peds.2009-3146

9 SOCIAL AND ENVIRONMENTAL HISTORY

This study will explore ancestry, geography, and environment. The following sections will provide in-depth insight into these areas while ensuring our results are generalizable across

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populations:

9.1 Maternal Risk Factors During Pregnancy

Exposure to maternal risk factors during pregnancy may affect the development of the fetus in-utero and after birth. Any developmental differences may affect what we see in the multiplexed-immunofluorescent images, single-cell sequencing, and other data that we are producing from biopsies and blood collected from participants.

Anemia during pregnancy is mostly due to iron deficiency and is associated with intrauterine growth retardation, prematurity, fetoplacental miss ratio, and higher risk for peripartum blood transfusion (James). Maternal iron deficiency can also lead to abnormal brain development in the fetus, leading to cognitive deficits in childhood (Georgieff et al).

Gestational diabetes increases the risk of long-term complications — including obesity, impaired glucose metabolism, and cardiovascular disease — in both the mother and the infant. Exposure to smoke or tobacco in other forms during pregnancy is associated with an increased risk of obstetric complications and adverse health outcomes for children exposed in-utero, while alcohol use as been associated with similar complications as well as a specific constellation of birth defects and intellectual and neurodevelopmental disabilities known as fetal alcohol spectrum disorders (Williams et al).

A	B	C	D
Anemia	☐ Yes	☐ No	☐ Unknown
Gestational diabetes	☐ Yes	☐ No	☐ Unknown
Use of tobacco products	☐ Yes	☐ No	☐ Unknown
If yes, select all that apply	☐ Chewable Tobacco		
	☐ Cigarettes		
	☐ Water pipe/Hookah		
	☐ Smokeless Tobacco (Snuff/Snus)		
	☐ e-Cigarettes/Vaping		
	☐ Other		
Alcohol use	☐ Yes	☐ No	☐ Unknown

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James AH (2021). Iron Deficiency Anemia in Pregnancy.. Obstet Gynecol..
http://10.1097/AOG.0000000000004559

Georgieff MK (2014). Iron deficiency in pregnancy. Am J Obstet Gynecol.
http://doi: 10.1016/j.ajog.2020.03.006

McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P (2019). Gestational diabetes mellitus.. Nature reviews. Disease primers.
https://doi.org/10.1038/s41572-019-0098-8

Williams JF, Smith VC (2015). COMMITTEE ON SUBSTANCE ABUSE. Fetal Alcohol Spectrum Disorders.. Pediatrics.
http://10.1542/peds.2015-3113

9.2 Breastfeeding History

World Health Organization called *Indicators for assessing infant and young child feeding practices (IYCF)* for ages 2 or younger (World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), 2021) includes indicators for breastfeeding. According to the CDC, infants who are breastfed have reduced risks of asthma, obesity, type 1 diabetes, severe lower respiratory disease, sudden infant death syndrome, gastrointestinal infections (diarrhea/vomiting), and necrotizing enterocolitis, and mothers who breastfeed have lower risks of hypertension, type 2 diabetes, and ovarian and breast cancers. However, due to a variety of factors, breastfeeding is not feasible in some situations. Thus, it is important to obtain a comprehensive history of breastfeeding to assess nutrition.

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A	B	C	D
Was the participant ever breastfed?	☐ Yes	☐ No	☐ I don't know
If yes, How long after birth was the participant first breastfed?	☐ Immediately	☐ ____ Hours	☐ ____ Days
If yes, In the first two days after delivery, was the participant given anything other than breastmilk to eat or drink (e.g. water, infant formula, goat milk, cow milk, juice)?	☐ Yes	☐ No	☐ I don't know
If yes, When did the participant last breastfeed?			
When was the participant first given solid foods?	☐ 0-3 months		
	☐ 3-6 months		
	☐ 6-9 months		
	☐ 9-12 months		
	☐ 12+ months		
	☐ I don’t know		

9.3 Demographic information about mother and/or father

For ancestry analysis, we would like to collect demographic information from the parents and grandparents of the patient. Categories analogous to those used for the patient are used for racial and/or ethnic information. Additional demographic information were adapted from the National Health and Nutrition Examination Survey (NHANES) a program of studies designed to assess the health and nutritional status of adults and children in the United States.The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions. These questions give us further insight into the environment of study participants. Additional questions were adapted from the 2020 United States Census questionnaire.

A	B	C
	Parent 1	**Parent 2**
Parent relationship to the participant	☐ Biological Mother	☐ Biological Mother
	☐ Non-Biological Mother	☐ Non-Biological Mother

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	☐ Non-Biological Father	☐ Non-Biological Father
	☐ Parent/Legal Guardian	☐ Parent/Legal Guardian
	☐ Other: _____	☐ Other: _____
Marital Status	☐ Married	☐ Married
	☐ Widowed	☐ Widowed
	☐ Divorced	☐ Divorced
	☐ Separated	☐ Separated
	☐ Single (Never married)	☐ Single (Never married)
Year of birth?		
If year of birth unknown, current age?		
Country of birth?		
If not born in present country, estimated date of arrival in present country? (DD-MMM-YYYY):		
	☐ Unknown or Does not apply	☐ Unknown or Does not apply
Primary language spoken at home?	☐ English	☐ English
	☐ Other:	☐ Other:
Highest level of education	☐ No Formal Schooling	☐ No Formal Schooling
	☐ Some Primary Education	☐ Some Primary Education
	☐ Primary Education	☐ Primary Education
	☐ Some High School (No diploma)	☐ Some High School (No diploma)
	☐ High School diploma or equivalent (e.g., GED)	☐ High School diploma or equivalent (e.g., GED)
	☐ Some college (No degree)	☐ Some college (No degree)
	☐ Undergraduate degree (Associate or Bachelor's)	☐ Undergraduate degree (Associate or Bachelor's)
	☐ Graduate or Professional degree	☐ Graduate or Professional degree
	☐ Trade School/Technical degree	☐ Trade School/Technical degree
Occupation?		
Total household income	☐ $0-$23,000	
	☐ $23,001-49,000	
	☐ $49,001-78,000	
	☐ $78,001-117,000	
	☐ $117,001-322,000	

Citation: Fatima Zulqarnain, Stephanie Regis, elsy.ngwa, Asra Usmani, jason.boisvert, Arg7Ef, Sana Syed, Jocelyn Silvester, jay.thiagarajah Participant Registration Form For Mapping the Early Childhood Gut Across Ancestry, Geography, and Environment (Gut-AGE) https://dx.doi.org/10.17504/protocols.io.6qpvr4d3zgmk/v1

	☐ More than $322,000	
	☐ Decline to answer	
Select all identities that apply, if there are additional identities not described below, please self-describe using "Not aligned with above categories" and the blank space provided.		
	Biological Mother:	**Biological Father:**
What is your race and/or ethnicity? Select all that apply	☐ Native American/Alaska Native	☐ Native American/Alaska Native
	☐ Black or African-American	☐ Black or African-American
	☐ Middle Eastern/North African	☐ Middle Eastern/North African
	☐ East Asian (e.g. Chinese, Japanese, Korean)	☐ East Asian (e.g. Chinese, Japanese, Korean)
	☐ Southeast Asian (e.g. Vietnamese, Filipino)	☐ Southeast Asian (e.g. Vietnamese, Filipino)
	☐ South Asian (e.g. Indian, Pakistani)	☐ South Asian (e.g. Indian, Pakistani)
	☐ White	☐ White
	☐ Native Hawaiian or Other Pacific Islander	☐ Native Hawaiian or Other Pacific Islander
	☐ Hispanic or Latino	☐ Hispanic or Latino
	☐ Not aligned with the above categories: _____ (e.g., Ashkenazi Jewish, French Canadian, Afro-Caribbean, etc.)	☐ Not aligned with the above categories: _____ (e.g., Ashkenazi Jewish, French Canadian, Afro-Caribbean, etc.)
	☐ Unknown	☐ Unknown
Family's self-identified racial and/or ethnic background?	**Biological Maternal Grandmother:**	**Biological Paternal Grandmother:**
	☐ Native American/Alaska Native	☐ Native American/Alaska Native
	☐ Black or African-American	☐ Black or African-American

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	☐ Middle Eastern/North African	☐ Middle Eastern/North African
	☐ East Asian (e.g. Chinese, Japanese, Korean)	☐ East Asian (e.g. Chinese, Japanese, Korean)
	☐ Southeast Asian ((e.g. Vietnamese, Filipino)	☐ Southeast Asian ((e.g. Vietnamese, Filipino)
	☐ South Asian (e.g. Indian, Pakistani)	☐ South Asian (e.g. Indian, Pakistani)
	☐ White	☐ White
	☐ Native Hawaiian or Other Pacific Islander	☐ Native Hawaiian or Other Pacific Islander
	☐ Not aligned with the above categories: _____ (e.g., Ashkenazi Jewish, French Canadian, Afro-Caribbean, etc.)	☐ Not aligned with the above categories: _____ (e.g., Ashkenazi Jewish, French Canadian, Afro-Caribbean, etc.)
	☐ Unknown	☐ Unknown
	Biological Maternal Grandfather:	**Biological Paternal Grandfather:**
	☐ Native American/Alaska Native	☐ Native American/Alaska Native
	☐ Black or African-American	☐ Black or African-American
	☐ Middle Eastern/North African	☐ Middle Eastern/North African
	☐ East Asian (e.g. Chinese, Japanese, Korean)	☐ East Asian (e.g. Chinese, Japanese, Korean)
	☐ Southeast Asian ((e.g. Vietnamese, Filipino)	☐ Southeast Asian ((e.g. Vietnamese, Filipino)
	☐ South Asian (e.g. Indian, Pakistani)	☐ South Asian (e.g. Indian, Pakistani)
	☐ White	☐ White
	☐ Native Hawaiian or Other Pacific Islander	☐ Native Hawaiian or Other Pacific Islander
	☐ Hispanic or Latino	☐ Hispanic or Latino
	☐ Not aligned with the above categories: _____ (e.g., Ashkenazi Jewish, French Canadian, Afro-Caribbean, etc.)	☐ Not aligned with the above categories: _____ (e.g., Ashkenazi Jewish, French Canadian, Afro-Caribbean, etc.)
	☐ Unknown	☐ Unknown

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10 PERCEIVED STRESS SCALE

The Perceived Stress Scale (PSS) was developed in 1983 by Cohen et al, and is a self-reported questionnaire designed to measure “the degree to which individuals appraise situations in their lives as stressful” (Cohen et al). The questionnaire generally assesses the degree to which the respondents believe their life has been overwhelming but does not focus on specific events or experiences. The 4-item PSS was adopted from the original 14-item scale, and the respondents answer each question on a five-point scale: never, almost never, sometimes, often, and very often.

Although the four-item PSS (PSS-4) has a moderate loss in internal reliability in comparison to the 14-item scale (r= 0.60 vs$r$= 0.85), the brevity of this instrument lends itself well to this study as we are already administering quite a lengthy questionnaire.

A	B
PERCEIVED STRESS SCALE	
We would like to ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate how often you felt or thought a certain way.	
In the last month, how often have you felt you were unable to control the important things in your life?	☐ Never
	☐ Almost Never
	☐ Sometimes
	☐ Often
	☐ Very Often
In the last month, how often have you felt confident about your ability to handle your personal problems?	☐ Never
	☐ Almost Never
	☐ Sometimes
	☐ Often
	☐ Very Often
In the last month, how often have you felt that things were going your way?	☐ Never
	☐ Almost Never
	☐ Sometimes
	☐ Often
	☐ Very Often
In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	☐ Never
	☐ Almost Never
	☐ Sometimes
	☐ Often
	☐ Very Often

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Cohen S., Kamarck T., Mermelstein R. (1983). A Global Measure of Perceived Stress. Journal of Health and Social Behavior, Vol. 24, No. 4, pp. 385-396.
http://396. 10.2307/2136404

Cohen S., Williamson G. ((1988). Perceived stress in a probability simple of the United States. The Social Psychology of Health.

11 HOUSEHOLD COMPOSITION

Similar to Step 9.3, some of the questions in this section were adapted from the National Health and Nutrition Examination Survey (NHANES) a program of studies designed to assess the health and nutritional status of adults and children in the United States. The NHANES interview includes demographic, socioeconomic, dietary, and health-related questions. These questions give us further insight into the environment of study participants. Additional questions were adapted from the 2020 United States Census questionnaire.

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A	B	C	D	E
HOUSEHOLD COMPOSITION				
Does the participant have any biological siblings?	☐ Yes	☐ No		
If yes, list year(s) of birth?				
How many individuals currently live in the participant's household?	Number of adults			
	Number of children			
Have you changed addresses since the participant was born?	☐ Yes	☐ No	☐ I don't know	☐ Decline to answer
If yes, how many times:	☐ 1			
	☐ 2			
	☐ 3			
	☐ 4+			
	☐ I don't know			
	☐ Decline to answer			
At what adddresses has the child lived at since birth?				
	Parent 1	**Parent 2**		
In what type of housing do you and your family currently reside in?	☐ Mortgage/own home	☐ Mortgage/own home		
	☐ Rental housing	☐ Rental housing		
	☐ Subsidized housing	☐ Subsidized housing		
	☐ Staying with friends/family	☐ Staying with friends/family		
	☐ Homeless/shelter	☐ Homeless/shelter		
	☐ Other:	☐ Other:		
What is the source of your drinking water?	☐ Well Water (Private)	☐ Well Water (Private)		
	☐ Tap/Public Utility	☐ Tap/Public Utility		
	☐ Bottled	☐ Bottled		
	☐ Community Well	☐ Community Well		
	☐ Other:	☐ Other:		

Centers for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Questionnaire (or Examination Protocol, or Laboratory Protocol). Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention

Citation: Fatima Zulqarnain, Stephanie Regis, elsy.ngwa, Asra Usmani, jason.boisvert, Arg7Ef, Sana Syed, Jocelyn Silvester, jay.thiagarajah Participant Registration Form For Mapping the Early Childhood Gut Across Ancestry, Geography, and Environment (Gut-AGE) https://dx.doi.org/10.17504/protocols.io.6qpvr4d3zgmk/v1

The 2020 Census questionnaire can be accessed at: https://www2.census.gov/programs-surveys/decennial/2020/technical-documentation/questionnaires-and-instructions/questionnaires/2020-informational-questionnaire.pdf

12 FAMILY MEDICAL HISTORY

The family medical history gives us further information about diseases that occur in the patient's family that may put the patient at a higher risk of developing these diseases.

A	B	C
FAMILY MEDICAL HISTORY		
	Biological Mother:	**Biological Father:**
Autoimmune diseases	☐ Lupus	☐ Lupus
	☐ Rheumatoid Arthritis	☐ Rheumatoid Arthritis
	☐ Diabetes Mellitus	☐ Diabetes Mellitus
	☐ Thyroid Condition	☐ Thyroid Condition
	☐ Psoriasis	☐ Psoriasis
	☐ Other:	☐ Other:
Gastrointestinal conditions		
Inflammatory Bowel Disease (Crohn's Disease or Ulcerative Colitis)	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Irritable Bowel Syndrome	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Celiac disease	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Eosinophilic Esophagitis (EoE)	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
H. pylori Gastric Infection	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
Other Gastrointestinal Disease:	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown
If yes, list:		
Other disorders		
Cancer	☐ Yes ☐ No ☐ Unknown	☐ Yes ☐ No ☐ Unknown

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