* 1. The 2020 study by Kortekangas et al.1 examined the effects of adverse environmental exposures on the gut microbiota composition of children and their mothers, an underexplored topic when compared to the impacts of gut microbiota on child health. The authors hypothesized that in mothers and their children at 18 months and 30 months of age, higher levels of adverse environmental exposures would be associated with lower microbiota diversity and maturity.
  2. The study design was an observational cohort study with prospective follow-up of participants. In this cohort study, there was a temporal aspect and there was longitudinal follow-up. Given the study question, this was an appropriate and strong study design since causality could be established due to the temporal aspect and longitudinal follow-up. Of note, the parent study of this paper (the iLiNS-DYAD trial) was a randomized nutritional intervention study. However, the randomized nutritional intervention was not studied in this paper and environmental exposures were only observed (not assigned) by investigators.
  3. The source population was pregnant participants of the iLiNS-DYAD trial in southern rural Malawi. From the 1,391 women enrolled in the main study, 869 had complete follow-up over the cohort time period. Of these women, stool samples were collected from only 343 mothers 1 month after delivery. From these 343 mothers, stool samples were available from 631 children at 18 months of age, and from 579 children at 30 months of age; all of these stool samples were used in analysis. The retention rate of children between the two time points was high; most of the participant exclusion for mothers and children was due to missed fecal sample collection.
  4. The primary environmental exposures were: below-median household assets index, household animal presence, lacking piped drinking water, sanitary facility, or a regular pit latrine, below-median maternal education, maternal HIV, and non-married maternal marital status. These exposures were directly measured except for the household assets index which is a well-established surrogate measure for socioeconomic status.2 The primary outcome variable was child microbiota maturity and diversity at ages 18 and 30 months. Secondary outcomes included child microbiota maturity and diversity at 1, 6, and 12 months and mothers’ microbiota outcomes in mothers 1 month postpartum. The gut microbiota was assessed via 16s rRNA sequencing of fecal samples; this is a surrogate measure of the mucosal large intestine microbiota. The use of surrogate measures necessitated a validity study to ensure that the fecal samples were good proxies for studying the gut microbiota. Yet, the authors did not perform any such studies. Collecting child fecal samples at multiple time points helped reduce within-person variability. However, samples were only collected from mothers once. This is a weakness, as within-person variability was not considered for the mothers. Fecal samples were collected via home visits, frozen at -20 ºC within six hours, frozen at -80ºC within 48 hours, then shipped for analysis on dry ice. DNA was isolated from fecal samples by bead-beating, then PCR was performed using V4 primers.1,3 OTUs were picked *de novo* and OTUs with <0.1% reads were removed. ASV and WGS were not discussed. Researchers ran linear models for associations between all exposures and microbiota maturity and diversity (using Shannon Index). Shannon Index is a strong measure of α-diversity, as it provides relative abundance quantitative information and accounts for rare species. However, the study would have been stronger with multiple α-diversity measures to compare findings. They used rigorous methods—weighted and unweighted UniFrac distances in PERMANOVA models—to test for β-diversity. Neither positive nor negative controls were utilized in this study, which could have validated analytical procedures. A source of measurement random error could be inter-technician variability, which has potential for information bias via non-differential misclassification of outcome. 16s may not have captured all bacterial species, so another validating method should have been used to prevent measurement systematic error and consequential information bias in the form of non-differential classification of outcome. Unfortunately, no variables were tested for reliability or validity in this paper. To control for confounders, the researchers adequately adjusted their models for exact gestational age and other exposures.
  5. The study found that low maternal education was associated with low child microbiota maturity and diversity. In children, season, water source, and maternal education was most associated with gut microbiota, and HIV status was the most important predictor for mothers’ relative OTU and genus abundance. High rates of follow-up, large sample size, and rigorous stool collection methods were strengths of the study. Limitations included no collection of dietary data, stress or inflammation, and household crowding. The strengths outweigh the limitations of the study as longitudinal follow-up allows causal interpretation. The results that maternal education and HIV status have implications for gut microbiome are biologically plausible. The results are generalizable to the rural Malawian population—because microbiota differ across populations, these results cannot be extended to other communities. Selection bias indicates systematic errors in study recruitment and causes internal invalidity. Generalizability is when study results can be applied to a target population, making the study externally valid. Policy implications of this study include advocating for women’s education, HIV testing and treatment, and safe water sources in Malawi, since these were some of the exposures linked to gut maturity and richness in the study population.