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Multigenerational adversity impacts on human gut microbiome composition and socioemotional functioning in early childhood

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Adversity exposures in the prenatal and postnatal period are associated with an increased risk for psychopathology, which can be perpetuated across generations. Nonhuman animal research highlights the gut microbiome as a putative biological mechanism underlying such generational risks. In a sample of 450 mother–child dyads living in Singapore, we examined associations between three distinct adversity exposures experienced across two generations—maternal childhood maltreatment, maternal prenatal anxiety, and second-generation children's exposure to stressful life events—and the gut microbiome composition of second-generation children at 2 y of age. We found distinct differences in gut microbiome profiles linked to each adversity exposure, as well as some nonaffected microbiome features (e.g., beta diversity). Remarkably, some of the microbial taxa associated with concurrent and prospective child socioemotional functioning shared overlapping putative functions with those affected by adversity, suggesting that the intergenerational transmission of adversity may have a lasting impact on children's mental health via alterations to gut microbiome functions. Our findings open up a new avenue of research into the underlying mechanisms of intergenerational transmission of mental health risks and the potential of the gut microbiome as a target for intervention.

intergenerational transmission | early life adversity | socioemotional functioning | gut microbiome | early childhood

Significance

This study draws on a large longitudinal cohort to demonstrate that adversity experienced prenatally or during early childhood, as well as adversity experienced by the mother during her childhood, impacts the gut microbiome of second-generation children at 2 y old. Notably, some of the microbiome profiles linked to these types of adversity, especially at higher taxonomic levels, were similar to those associated with the child's current and future socioemotional functioning. Additionally, microbes uniquely associated with adversity exposures or socioemotional functioning have similar immune-related functions within the gut, highlighting the need for further research into how generational adversity affects the gut microbiome's functional potential.

Adversities such as maltreatment or parental mental illness that are experienced in fetal or early postnatal life are strongly linked to emerging psychopathology (1). Those elevated mental health risks carry forward into the next generation (2). Central and peripheral systems such as the brain and hypothalamic-pituitary-adrenal axis are biological conduits through which adversity can impact psychopathology across generations (3, 4). Preliminary research implicates the gut microbiome as another such conduit. The gut microbiome is highly plastic in early postnatal life (5), appears to be shaped by prenatal and postnatal experiences of adversity (6–10), and is causally connected to emotional health in adulthood (11). Knowing how the microbiome is linked to adversity within and across generations, and whether such changes undergird associated increases in psychopathology, could seed a wave of novel treatments and preventions for mental illness in adversity-exposed families.

Rodent studies demonstrate that prenatal stress disrupts the maternal vaginal and gut microbiomes, and consequently the gut microbiome of infants delivered vaginally (12, 13), an effect which can be detected even in adulthood (14). The gut microbiome of pregnant mothers has also been found to shape fetal brain and immune system development (15, 16), which could subsequently impact the child's microbiome through brain–gut–microbiome and immune–gut–microbiome communication pathways (17–19). In humans, prenatal adversity (maternal psychological distress) has been shown to impact the gut microbiome of infants measured shortly after birth (20, 21). However, no study in humans has investigated the relationship between prenatal adversity and gut microbiome composition beyond early infancy.

Direct exposure to adversity in postnatal life also shapes the developing gut microbiome. Maternal separation stress in infancy causes early emerging and long-lasting changes to gut microbial communities in rodents (22–24). Also in rodents, microbiome-based interventions administered in early life can reverse the impact of postnatal adversity on the microbiome and behavior (24–27). In humans, adversity occurring within the first 3 y of life has been associated with altered gut microbiome composition (7–9), though the studies are limited by small sample sizes (Ns = 16 to 48). Interestingly, adult women who reported higher childhood adversity had an altered

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microbiome composition relative to women with fewer of those experiences (10), suggesting that adversity impacts on the microbiome are long-lasting.

Intergenerational impacts of adversity on the microbiome have not been examined in humans, but compelling evidence exists in rodents. Specifically, affective behaviors were altered in second-generation rats whose fathers were exposed to early adversity, but this effect was ameliorated if the fathers themselves or the second-generation offspring were treated with a probiotic. (27). These data suggest that alterations to the microbiome produced by adversity may be transmitted to the second generation and could be sufficient (if not necessary) for the intergenerational impact of that experience on affective behavior. As the microbiome has been linked to emotional behaviors and associated neural circuits in human children (6, 7, 28–30), examining the impacts of direct and intergenerational adversities on the human microbiome will inform future interventions targeted at ameliorating psychopathology.

In the current study, we used a large sample of mother–child dyads to investigate the impacts of maternal childhood maltreatment, maternal prenatal anxiety, and children's exposure to stressful life events on the gut microbiome at child age 2 y. The first 2 to 3 y of life are a period of rapid brain–gut–microbiome development, and changes to the microbiome during that time are proposed to shape lifetime risk for psychiatric disorders (31–33). To test the behavioral relevance of adversity-associated microbiome characteristics we then examined links between second-generation children's gut microbiome at 2 y of age and their socioemotional functioning at 2 and 4 y of age. Based on past research showing associations between childhood maltreatment, maternal prenatal stress, and child stress with bacterial taxa associated with inflammation (e.g., *Prevotella*; 8, 10, 21), we hypothesized that all adversities would be associated with higher abundance of bacteria associated with inflammation. As only postnatal adversity has previously been associated with differences in alpha diversity, we hypothesized a specific effect of children's exposure to stressful life events on lower alpha diversity at 2 y of age. Finally, because cumulative adversity has been found to impact childhood psychopathology symptoms more than single and more time-limited exposures (34–36), we hypothesized that increasing timepoints of adversity exposure would be associated with a higher abundance of proinflammatory bacteria, and potentially lower alpha diversity.

Results

Gut Microbiome Alpha Diversity Is Associated with Prenatal and Postnatal Adversity Exposures. Controlling for selected covariates, there was a significant direct effect of postnatal adversity exposure (over and above preconception and prenatal adversity) on Faith's phylogenetic diversity (PD), with greater postnatal adversity associated with lower diversity ($\beta = -0.31$, $P = 0.038$, $\Delta R^2 = 0.014$; Fig. 1). We also found significant direct ($\beta = 0.16$, $P = 0.034$, $\Delta R^2 = 0.024$; Fig. 1) and total [$\beta = 0.13$, 95% CI = (0.01, 0.25), $\Delta R^2 = 0.02$] effects of prenatal adversity exposure on Pielou evenness (*SI Appendix*, Fig. S6). No other significant associations between adversity exposures and alpha diversity metrics were found (see *SI Appendix*, Tables S9 and S10 for full regression results and *SI Appendix*, Figs. S4–S7 for serial mediation models). Results without including covariates were very similar (*SI Appendix*, Tables S14). Controlling for selected covariates, no significant differences in alpha diversity as a function of cumulative adversity were found (*SI Appendix*, Table S11); results without covariates were almost identical (*SI Appendix*, Table S15).

Gut Microbiome Beta Diversity Is Not Associated with Adversity Exposure. Controlling for selected covariates, none of the adversity exposure variables explained significant variance

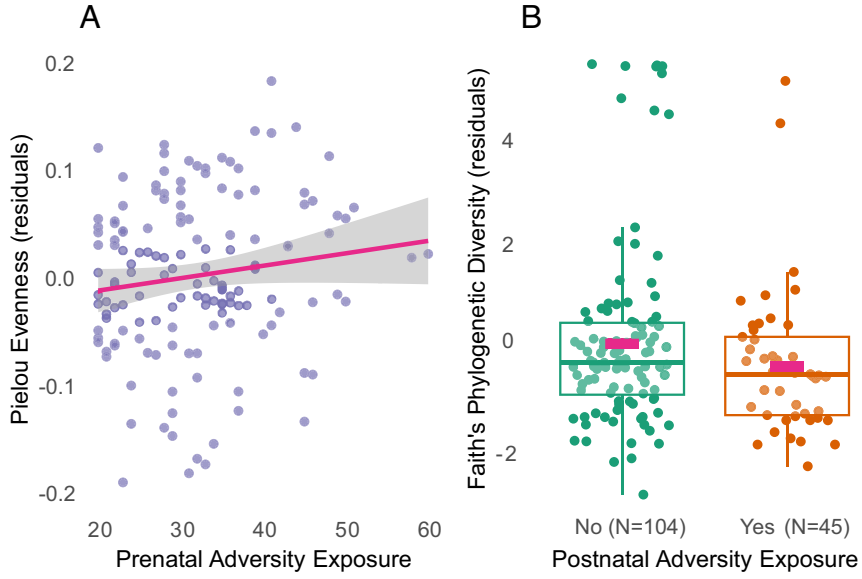

Fig. 1. Significant associations between adversity exposure and alpha diversity. Each dot represents a participant. Alpha diversity values are residuals after partialling out variance accounted for by covariates. Predictors in the models include preconception adversity, prenatal adversity, postnatal adversity, monthly income per household member, child sex, microbiome sequencing batch, fiber, polyunsaturated fatty acids (PUFA), and delivery mode. (*A*) Pielou Evenness is higher among children with more prenatal adversity exposure. Prenatal adversity exposure was measured as mothers' state anxiety score during pregnancy. Grey shaded area around the line is the 95% CI level for the predicted best-fit function using a linear model. (*B*) Dots have been jittered along the x axis to increase visibility of individual data points. Faith's PD is lower among children with more postnatal adversity exposure. Postnatal adversity exposure ("Yes") indicates at least one potentially stressful life event reported between child's birth and age 2 y; no postnatal adversity exposure ("No") indicates no potentially stressful events reported. Thick horizontal pink lines indicate the mean for each exposure group. Boxplot represents the median (line in the middle of the box), upper 25% quantile (top of the box), lower 25% quantile (bottom of the box), upper 25% quantile minus 1.5 times the interquartile range (upper whisker), and lower 25% quantile minus 1.5 times the interquartile range (lower whisker) Faith's PD values for each group.

in any of the beta diversity distance matrices (*SI Appendix*, Table S12). Results without covariates were similar (*SI Appendix*, Table S16). Controlling for selected covariates, cumulative adversity did not explain significant variance in any of the beta diversity distance matrices (*SI Appendix*, Table S13); results without covariates were similar (*SI Appendix*, Table S17).

Abundance of Several Gut Microbiome Taxa Differs According to Adversity Exposures and Socioemotional Functioning Outcomes. ***Each adversity is associated with abundance of distinct taxa in separate models for each exposure.*** Controlling for selected covariates, one distinct bacterial taxon was differentially abundant as a function of preconception adversity (from genus *Clostridium sensu stricto*, positively associated with adversity), three as a function of prenatal adversity (two from genus *Streptococcus* positively associated with adversity and one from genus *Ruminococcus* negatively associated with adversity), and two as a function of postnatal adversity (one from genus *Parabacteroides* negatively associated with adversity and one from genus *Finegoldia* positively associated with adversity; Fig. 2 and *SI Appendix*, Table S19). Results without covariates were the same for preconception and prenatal adversity; no taxa were differentially abundant as a function of postnatal adversity when covariates were excluded (*SI Appendix*, Table S26).

Prenatal and postnatal adversities are uniquely associated with taxa abundance. Controlling for selected covariates, and including all adversity exposures in the same model, one distinct taxon was less abundant with more prenatal adversity exposure (from *Ruminococcus* genus), and one was less abundant with more postnatal adversity exposure (from genus *Parabacteroides;* Fig. 2 and *SI Appendix*, Table S18). Results without covariates were the same for prenatal adversity; for postnatal adversity, there were no differentially abundant taxa (*SI Appendix*, Table S25).

Cumulative adversity is not associated with taxa abundance. No covariates were selected; cumulative adversity was not associated with the abundance of any taxa.

Preconception adversity subtypes are associated with abundance of similar and distinct taxa. Controlling for selected covariates, taxa from the genus *Clostridium sensu stricto* were more abundant with more exposure to each adversity subtype, with sexual abuse being most strongly associated. One additional taxon was more abundant with more physical abuse (from genus *Bfidobacterium*), two were more abundant with more physical neglect (from genera *Anaerofustis* and *Ezakiella*), and two were more abundant with more emotional abuse (from genera *Lachnoclostridium* and *Lactococcus*; *SI Appendix*, Table S20).

Adversity-associated child socioemotional functioning outcomes are associated with abundance of distinct taxa in separate models for each outcome. Controlling for selected covariates and for correlated adversity exposures, one distinct taxon (from genus *Intestinibacter*) was less abundant with more total problems at age 2 y and with more internalizing problems at age 4 y, one was less abundant with more developmental problems at age 2 y (from genus *Streptococcus*), and five were differentially abundant as a function of sleep problems at age 4 y (from *Coprobacillus*, *Lachnospiraceae UCG-8*, and *Faecalibacterium* negatively associated with sleep problems; from *Veillonella*, and *Blautia* positively associated with sleep problems; Fig. 3 and *SI Appendix*, Table S21). One of these results overlapped with adversity findings: abundance of taxa from the *Streptococcus* genus was negatively associated with developmental problems at age 2 y and positively associated with prenatal adversity. Without controlling for covariates, results were similar for total problems

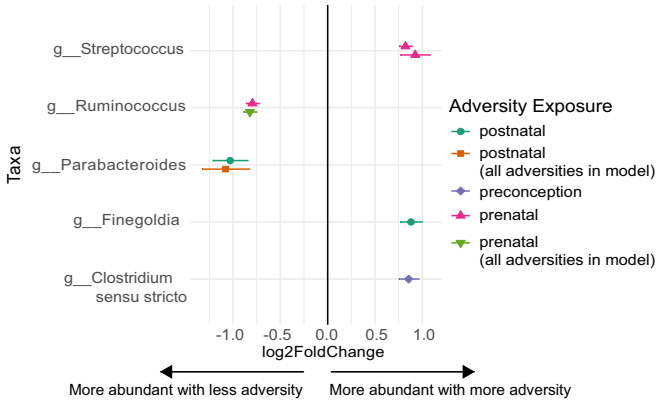

Fig. 2. Differentially abundant taxa as a function of adversity exposure. Dots represent base 2 log fold change in abundance with a standardized one-unit increase in adversity exposure (i.e., each coefficient estimate converted to log2FoldChange). Horizontal lines surrounding the dots represent the 95% CI around each coefficient estimate, also converted to log2FoldChange. g__ indicates each taxon's name is defined to the genus level. Taxa shown here met criteria for significance at $q < 0.25$ after false discovery rate (FDR) correction. Only those associations that had nonzero abundance of the differentially abundant taxon in at least 15% of the total sample are shown here; associations with lower nonzero abundance are presented in *SI Appendix*, Table S13. Results are shown for preconception, prenatal, and postnatal adversity, and also for each of prenatal and postnatal adversity when accounting for the variance attributed to the other adversities (all adversities in model). Covariates include child ethnicity (all analyses), duration of exclusive breastfeeding and sequencing batch (preconception, prenatal, and all adversities in model), probiotic use (prenatal adversity), and child age at stool collection (all adversities in model).

at age 2 y and internalizing problems at age 4 y; no taxa were differentially abundant as a function of developmental problems at age 2 y, and only two of the taxa (from *Lachnospiraceae UCG-8* and *Blautia* genera) were differentially abundant as a function of sleep problems at age 4 y (*SI Appendix*, Table S27).

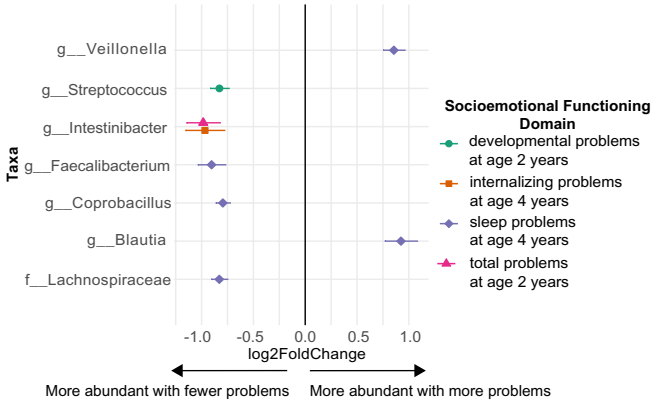

Fig. 3. Differentially abundant taxa as a function of adversity-related child socioemotional functioning. Dots represent base 2 log fold change in abundance with a standardized one-unit increase in socioemotional functioning problems (i.e., each coefficient estimate converted to log2FoldChange). Horizontal lines surrounding the dots represent error bars which are the 95% CIs around each coefficient estimate, also converted to log2FoldChange. g__ or f__ indicates each taxon's name is defined to the genus or family level, respectively. Taxa shown here met criteria for significance at $q < 0.25$ after FDR-correction. Only those associations that had nonzero abundance of the differentially abundant taxon in at least 15% of the total sample are shown here. Other predictors include child sex and prenatal adversity (all analyses), preconception adversity (internalizing problems age 2 y and total problems age 4 y), child ethnicity and duration of exclusive breastfeeding (developmental problems age 2 y, internalizing and sleep problems age 4 y), microbiome sample sequencing batch (total problems age 2 y, internalizing and sleep problems age 4 y), fiber (internalizing problems age 4 y) and probiotic use (sleep problems age 4 y).

Discussion

This study demonstrated intergenerational and direct exposure impacts of adversity on the gut microbiome of 2-y-old children, and also showed that child microbiomes were related to behavior. We found distinct taxa that were differentially abundant as a function of each adversity, with no overlap between the adversities. This suggests that exposure to adversity at different stages of life (preconception, prenatal, postnatal) each have a distinct impact on the gut microbiome composition of 2-y-old children. Moreover, when accounting for the impact of other adversity exposures, we saw that there were unique effects of prenatal and postnatal adversities, but not of preconception adversity on differentially abundant taxa. This suggests that preconception adversity may impact the abundance of specific microbial taxa of second-generation youth via its association with prenatal and postnatal adversity exposures.

While distinct microbiome taxonomic profiles were observed across adversities and with behavior, inferred functional associations overlapped (37). Specifically, an inefficient butyrate producer, *Clostridium sensu stricto*, was more abundant among children with higher preconception adversity (regardless of adversity subtype), whereas a more efficient butyrate producer (*Ruminoccocus*) was less abundant among children with prenatal adversity. In contrast, prenatal adversity, postnatal adversity, and child socioemotional problems were associated with increased abundance of inflammation-associated taxa: *Finegoldia* (postnatal adversity) and *Streptoccocus* (prenatal adversity), and decreased abundance of anti-inflammatory associated taxa: *Parabacteriodes* (postnatal adversity) (38–41), and *Intestinibacter* (total problems at 2 y of age) (42). This regulation of inflammation-associated bacteria by adversity exposure is consistent with past research in older age groups (8, 10).

One unexpected result was that some anti-inflammatory taxa, *Blautia* and *Veillonella* (43, 44), were associated with poorer child socioemotional functioning at 2 y of age. However, these results are consistent with prior research showing positive associations between these taxa and sleep problems (45, 46), depressive symptoms (47), and externalizing symptoms (6). These results may be due to functional differences within the species or strains contained within the genus that cannot be resolved with 16S amplicon sequencing. Future studies should use whole genome approaches to establish functional potential of the microbiome directly.

Interestingly, and consistent with past research (7, 8), many of the taxa associated with each adversity exposure, and with child socioemotional outcomes, were from the order Clostridiales. That so many differentially abundant genera fell into the Clostridiales order suggests that this order may be especially stress reactive. Targeting this order may be fruitful for future interventions to reduce transdiagnostic risks for socioemotional health problems following adversity exposure.

Beyond the differential abundance of taxa, adversity was also associated with intraindividual community composition of the gut microbiome in early childhood. Consistent with some past research (7, 48, 49), and our hypotheses, we saw that postnatal adversity was associated with lower phylogenetic alpha diversity of the microbiome. However, inconsistent with past research (8), and our hypotheses, prenatal adversity was associated with greater evenness of taxa within the gut; although even taxa distribution is often associated with good health outcomes in adults (50), it is unclear whether the same is true of children (51).

It is important to note that some of our results, especially those on differential abundance, were no longer significant when covariates were excluded. Potential covariates are numerous in microbiome research and, when associated with the outcome variable but not the predictor of interest, as was the case for our impacted analyses (*SI Appendix*, Table S6), typically increase power by accounting for residual variance in the outcome. Thus, transparency with covariate selection, and reporting outcomes with and without covariates included is imperative for future microbiome research (52).

While we were most interested in the unique effects of adversities on the gut microbiome, we also characterized associations between cumulative adversity (i.e., substantial exposure to adversity at 0, 1, or 2+ timepoints) and gut microbiome composition. Unexpectedly, we saw no associations between cumulative adversity and any of the investigated microbiome features, nor with any child socioemotional functioning variables. However, within our community sample, relatively few families fell into the highest accumulation group which could have rendered us underpowered to detect those effects.

There were several limitations to our study. Given the nature of the data we could not determine if differences between the adversities were related to the type or timing of exposure. Also, the questionnaires used to assess adversity have not been validated in Singapore, though they have been validated within the dominant cultural group in Singapore–Chinese (53). As the majority of the sample was of Chinese ethnicity, and findings were highly similar when analyses were repeated within the Chinese subsample only, caution is warranted in generalizing the findings beyond Chinese (i.e., to Malay and Indian) populations. Finally, we were only able to examine one "-omics" layer within the gut (i.e., the genome). Multiomics approaches, e.g., metabolome and genome, may provide better insight into the complex biological processes at play following adversity exposure (54).

In conclusion, these data show that adversity experienced directly or intergenerationally can influence the microbiome during a period of maximal developmental change—the first 2 y of life. Moreover, our data highlight that the influence of adversity on the microbiome–immune pathway is a likely biological conduit through which adversity impacts child socioemotional development.

Materials and Methods

Participants and Study Design. Participants were women aged 18 y and above who enrolled in the Growing Up in Singapore Towards Healthy Outcomes (GUSTO) study during their second trimester antenatal dating ultrasound appointment in one of two major maternity hospitals in Singapore, and their child (55). The GUSTO study was approved by the National Healthcare Group Domain Specific Review Board and the SingHealth Centralised Institutional Review Board in Singapore. All women provided informed written consent for themselves and their children.

The current sample includes 450 mother-child dyads for whom the child donated a stool sample (for microbiome analysis) at 2 y of age, and who had data from at least one of three adversity measures: preconception adversity (285 dyads), prenatal adversity (440 dyads), and postnatal adversity (309 dyads); 205 dyads provided usable data across all adversity assessments. See *SI Appendix* for *Descriptive Statistics*, *Exclusion Criteria*, and *Stool Sample Collection* information. *SI Appendix*, Fig. S1 illustrates the data collection timeline for adversity, SEF (socioemotional functioning), and gut microbiome measures.

Measures.

Preconception adversity. Mothers retrospectively reported on their own history of childhood maltreatment using the Childhood Trauma Questionnaire-Short Form (CTQ-SF; 56).

Prenatal adversity. At 26 to 28 wk gestation, mothers reported on their current anxiety levels using the state subscale of the State-Trait Anxiety Inventory, Form Y (STAI-S; 57).

Postnatal adversity. Caregivers reported on potentially stressful life events experienced by the participating child via the Life Events Questionnaire (LEQ; 58). Because relatively few caregivers reported stressful events that had occurred before child age 2 y (age cutoff used to be concurrent with stool sample), we created a dichotomous variable: zero life events before age 2 y or 1 or more, to be used in further analyses.

Cumulative adversity. A cumulative adversity measure was calculated to quantify the number of timepoints (preconception, prenatal, postnatal) when each dyad

reported exposure to adversity (0, 1, or 2+ timepoints). Exposure cutoffs for each timepoint were designed to separate dyads who had substantial exposure to adversity from those who did not.

Child socioemotional functioning. Caregivers reported on their child's SEF at child ages 2 and 4 y using the Child Behavior Checklist version for children 1.5 to 5 y of age (CBCL; 59). A total problem score and 14 subscale scores were calculated that encompass different domains of socioemotional functioning (e.g., internalizing and externalizing problems).

Missing data. A cutoff specifying the percentage of items with missing responses above which the participant's dataset would be excluded was determined for each questionnaire according to scoring manual instructions: 20% missing on the CTQ-SF (preconception adversity), 10% missing on the STAI-S (prenatal adversity), and 10% missing on each socioemotional functioning domain on the CBCL. After excluding datasets with excessive missing data, any missing items were mean imputed before score calculation. There was no missing data on the LEQ event occurrence items (postnatal adversity), and thus no data were imputed for that measure. See *SI Appendix* for additional details on each measure and missing data mechanism.

Gut Microbiome Bioinformatics. Detailed descriptions of DNA extraction and sequencing can be found in ref. 59. In short, DNA was extracted using MoBio PowerFecal DNA kits (60). Sequencing was performed using the Illumina MiSeq platform standard protocol (61). Operational taxonomic unit (OTU) delineation was performed using USEARCH v9.2.64 at a 97% similarity threshold. Taxonomy was assigned to each OTU by comparing against the SILVA 123 ribosomal reference database (https://www.arb-silva.de/) (60). QIIME v2.0 (62) was used to normalize microbiome data using rarefaction (depth = 5,777), which accounts for uneven sequencing depth between samples (63 and *SI Appendix*, Fig. S2), and to compute alpha and beta diversity metrics. Alpha diversity (within-individual bacterial community diversity) indices included richness, or number of distinct taxa (observed features), relative evenness of taxa within the community (Pielou evenness), richness weighted by evenness (Shannon index), and genetic diversity (Faith's PD). Beta diversity indices included phylogenetic distance (weighted and unweighted Unifrac; (63), presence/absence similarity (Jaccard), and abundance similarity (Bray–Curtis) (64, 65).

Data Analysis.

Relationship between adversity exposure and alpha diversity. All three adversity exposures, or cumulative adversity, were entered into a series of ordinary least squares multiple regression models predicting alpha diversity metrics (controlling for selected covariates; see covariate selection section and *SI Appendix*, Tables S4 and S5 for a list of included covariates) using heteroskedasticity robust SEs (66). For cumulative adversity analyses, we entered two binary variables, 0 vs. 1 timepoint and 0 vs. 2 or more timepoints of exposure, as predictors into the models.

Direct and indirect effects of each timepoint of adversity exposure on alpha diversity. To quantify the indirect and total effects of prenatal and preconception adversity on alpha diversity, in addition to direct effects of each adversity exposure timepoint controlling for the others, we estimated a series of serial mediation models in Mplus version 8.3. One model was estimated for each alpha diversity metric, with weighted least squares estimation, probit models specified for equations with the binary postnatal adversity variable as the outcome, and theta parameterization (67). Significance for indirect effects was estimated based on 95% CIs with 1,000 bootstrapped samples (see *SI Appendix*, Figs. S4–S7 for a visualization of the models and *SI Appendix*, Table S10 for total effects model results).

Relationship between adversity exposure and beta diversity. Binarized adversity exposure variables (to improve interpretation; see *SI Appendix*, cumulative adversity section for details), or the three-category cumulative adversity variable, were entered into a series of PERMANOVA models predicting variance in each beta diversity distance matrix. Each analysis controlled for selected covariates (see *SI Appendix*, Tables S4 and S5 for a list of included covariates).

Differential taxa abundance as a function of adversity exposure. Differences in the relative abundance of each taxon as a function of adversity exposure were analyzed using MaAsLin2 on data filtered for nonzero abundance at 0.5% (keeping 773 taxa out of 1,230; results using more conservative –0.1%, and liberal –1%, thresholds are presented in the *SI Appendix*), controlling for selected covariates (see *SI Appendix*, Table S4 for covariate list) (68). In differential abundance analyses, the effective sample size is often substantially reduced due to high zero inflation within taxa counts. As a result, we performed differential abundance analyses entering all three adversity exposures into each MaAsLin2 analysis, as had been done with the alpha and beta diversity analyses (N = 196), and we also performed an additional set of MaAsLin2 analyses with adversity exposures entered separately to increase the sample size (N = 211 to 309). *P*-values were corrected for multiple comparisons using the Benjamin-Hochberg method, with a q value threshold for significance of 0.25, as has been used in prior work and recommended for biomarker discovery approaches (69). To highlight the most reliable results, significantly differentially abundant taxa that had nonzero abundance in at least 15% of the total number of included samples are discussed the main text, while the remaining significant results are reported in the *SI Appendix*. We also performed a set of follow-up exploratory analyses examining differential abundance as a function of preconception adversity subtypes using the same procedure.

Associations between adversity-related child socioemotional functioning and differential taxa abundance. Adversity-related child socioemotional variables were identified with bivariate Pearson correlations (for continuous variables), *t* tests (for binary variables) and ANOVAs (for multicategorical variables) between each category of adversity exposure and cumulative adversity and each child socioemotional functioning domain measured at 2 y and 4 y of age, using the Benjamin–Hochberg method of adjustment for multiple comparisons within each test method. MaAsLin2 was used to identify taxa whose relative abundance was associated with any of the adversity-related socioemotional functioning domains, controlling for the correlated adversity variable(s) and selected covariates. The final set of child socioemotional functioning domains used in these analyses, their selected covariates, and associated adversity exposures, is listed in *SI Appendix*, Table S4.

Covariate selection. As the number of potential covariates to include in analyses was large, we first selected subsets of covariates to use in each analysis by identifying those that explained significant variance in the outcomes of interest (see *SI Appendix* for list of possible covariates and *SI Appendix*, Fig. S2 and Tables S4 and S5 for covariate selection results). For analyses that included child socioemotional functioning, we also used child sex as an a priori covariate as there are differences in the mean and SDs of child socioemotional functioning domains between males and females. To examine whether our results were robust to the specific covariates included in each analysis, we report analyses without covariates in the *SI Appendix*. See *SI Appendix* for additional details on covariate selection and *SI Appendix*, Table S6 for relationships between covariates and predictor variables of interest.

Data, Materials, and Software Availability. All code and information on software versions used in this manuscript is available at: https://github.com/bablab/gusto_adversity_microbiome_SEF (70). The data supporting the findings of this research are publicly accessible using access procedures modeled after those of the NIH through requests to the GUSTO Executive Committee, of which C.Y.S., P.D.G., K.G., and M.J.M. are members. Requests should be directed to the corresponding authors.

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