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Mode of delivery and maternal body mass index are weakly associated with the infant gut microbiota composition

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SUMMARY Obesity has grown to epidemic proportions in many countries across the globe. The health risks associated with it has necessitated research studying potential determinants of childhood and adult obesity. Many factors such as mode of delivery and maternal body mass index have been identified to influence the infant gut microbiota and weight status. In this study, we sought to both validate the effects of these factors on infant gut microbiome composition and investigate how they affect early life development using an unpublished dataset collected from 325 infant-mother dyads. The dataset was generated by Dr. Kyung Rhee from UCSD and documented medical histories and early life infant growth and behaviour. Our results showed that infant delivery method and maternal BMI were associated with small taxonomic changes but not significant overall gut microbiome changes. Infant delivery method and maternal BMI also did not show statistically significant effects on infant early life weight gain trajectory. These findings suggest the mode of delivery and maternal BMI may have small-scale impacts on gut microbiome composition and weight gain for the cohort we studied.

INTRODUCTION

With over 650 million adults being classified as obese in 2016, obesity is one of the most prevalent contemporary health conditions, according to the World Health Organization (https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight). Recent studies have shown that overweight infants are more susceptible to obesity during adulthood (1). Infant gut microbial composition has been associated with infant obesity, and is in turn influenced by many factors (2–4). In particular, the mother is implicated to have significant impacts on infant gut microbiome through variables such as infant feeding method (5). Therefore, we wanted to study some of the maternal determinants of infant gut microbiota.

To investigate these factors, we studied a dataset that documented information including the medical histories, stool microbiome compositions, infant growth, and feeding behaviour of 325 infant-mother pairs. The dataset was generated by Dr. Kyung Rhee from UCSD and is available on the European Nucleotide Archive (ENA) Browser (https://www.ebi.ac.uk/ena/browser/view/PRJEB39437).

One variable of interest in this dataset was the mode of delivery. Delivery method has been well documented as a major determinant of the gut microbiome in infants during childbirth (6). Vaginal deliveries lead to infant gut being colonized by similar bacteria as maternal gut and vagina. Meanwhile, babies delivered via C-section have little to no vaginal or maternal gut bacteria at birth, instead being colonized by skin bacteria. Even after birth, babies delivered by C-section show delayed colonization of the gut by typical commensals (ex. *Bacteroides*, *Bifidobacterium*) (6). Differences in the gut microbiome as a result of mode of delivery have been previously linked with obesity, as well as other important health outcomes like early cognitive behavioral development (7, 8). Thus, we sought to determine whether other behaviors, specifically feeding behaviors, were similarly impacted by differences in mode of delivery. In addition, we hoped to confirm that mode of delivery differences could drive gut microbiome differences in the dataset we analyzed.

In addition to mode of delivery, Dr. Rhee's study also collected maternal body weight at times pre and post child delivery. Previous studies have shown maternal body mass index (BMI) before and during pregnancy to be a major predictor of adult obesity and was shown to be a better predictor of offspring BMI than paternal BMI (9, 10). Furthermore, a previous

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systems biology study on fecal metagenomes suggested obese gut microbiomes may lack diversity and have different gut microbiome-host interactions compared to those of lean gut microbiomes (11). Infant gut microbiomes are known to resemble those of their mothers, which suggests aberrant bacteria from obese maternal microbiomes can be passed from mother to child (12). This study aimed to observe differences between obese/overweight microbiomes and normal weight maternal microbiomes, similarities between maternal and infant microbiomes, and the effects of infant microbiome differences on infant weight-for-length Z scores.

Given the factors that affect the microbiota outlined above, this study aimed to investigate some of the maternal factors that may affect infant gut microbial composition and weight gain trajectory in the cohort developed by Dr. Kyung Rhee from UCSD. Specifically, we investigated the effects of infant delivery method and maternal BMI closest to birth. We predict maternal BMI and delivery methods will alter infant feeding behaviour, weight to length ratio, and gut microbiome.

METHODS AND MATERIALS

All codes for the procedure and details of metadata filtering can be found in the attached text files.

Dataset. In this paper, we used an unpublished dataset that was compiled by Dr. Kyung Rhee from UCSD with the goal of examining infant gut microbiome, early life feeding behaviour, and weight gain trajectory. The dataset provides information on infant-mother pairs that documents their medical history, weight gain trajectory, feeding behaviour and feeding method at different time points. Stool samples for microbiota analysis were collected from 325 mother-infant dyads at 2 weeks, 2 months, 4 months, 6 months, and 12 months. Microbial compositions were identified using 16S rRNA sequencing and fecal metabolomics. The dataset is available on the European Nucleotide Archive (ENA) Browser (https://www.ebi.ac.uk/ena/browser/view/PRJEB39437).

Early data analysis with QIIME. Dr. Rhee's dataset was demultiplexed thanks to Mihai Cirstea of the Microbiology and Immunology department of UBC. The data was imported into QIIME2 (13) using the semantic type 'SampleData[SequencesWithQuality]' for artifacts. The demultiplexed file was visualized and a high quality score (greater than 30) was observed for all 150 sequence bases. Sequence quality control was performed with DADA2 to determine amplicon sequence variants (ASV), where all 150 bases were preserved. The DADA2 and ASV statistics were visualized as qzv files. A sampling depth of 4250 was chosen based on a created alpha-rarefaction plot, as observed features plateaued and the number of samples decreased with increasing sampling depth after this point for the metadata category of "infant_wlz" (Fig. S1) and other categories (not shown). This sampling depth was used to generate a tree for phylogenetic analysis.

Classification of maternal BMI classes. Maternal BMI was classified according to the NIH classification by the United States Centre for Disease Control (https://www.nih.gov/news-events/news-releases/nih-study-identifies-ideal-body-mass-index). Infants were sorted into maternal weight categories (i.e. infants born to obese mothers). Weight categories defined by BMI can be found in the supplementary data (Table S1).

Correlation analyses for early life determinants. Correlation analyses were carried out using the metadata file. The data was subsetted as appropriate for each potential determinant (see attached text files). Averages over time or interquartile ranges of metadata categories were plotted as line or box plots, respectively, using ggplot2 in R (14). Two factor t-tests or pairwise comparisons using Tukey-Kramer tests were done to determine statistical significance of differences.

Beta-diversity analysis metrics. The analysis was done in both QIIME2 and R, using the tidyverse, vegan, phyloseq, DESeq2, and ggplot2 packages (15–17). To integrate the data from QIIME2, the metadata file was combined with the "table-with-taxonomy.biom" and

"tree.nwk" files outputted from QIIME2 into a physeq object in R. This was subsetted as appropriate for each potential determinant. Data was then rarified to a 4250 sampling depth and abundance counts were transformed to relative abundance. Relative abundance counts were analyzed using the weighted UniFrac measure since it captured the most variability between samples. Bray-Curtis, Jaccard, and unweighted UniFrac measures were also used to compare to results seen with weighted UniFrac. Results were plotted on PCoA plots.

Differential abundance analysis. The "table-with-taxonomy.biom" and "tree.nwk" files from QIIME2 were combined with the metadata file into a phyloseq object in R. Total ASVs across all samples were then used to determine relative abundance. To capture relative abundance differences, we pruned ASVs with a relative abundance cutoff at > 0.0001 and merged species at the family taxonomic rank. Using DESeq2, we calculated differential abundance and plotted the significant families with a false discovery rate (FDR) corrected p-value of 0.05. The analysis for maternal BMI as a potential determinant used a modified metadata file with additional columns for maternal BMI classifications. To create volcano plots, Analysis of Composition of Microbiomes (ANCOM) was done (18). In QIIME2, the "table.qza" file was subsetted into only infant samples, and features with a frequency of less than 10 were removed. The table was then collapsed to the genus level and converted to a composition artifact and ANCOM analysis was done on the composition artifact. Results were exported as a tsv and visualized in R using the ggplot2 package where customization of the plot was required.

RESULTS

Caesarean-section delivered infants trend towards increased feeding and weight-for-length z-scores. Previous literature reports that differences in mode of delivery have been associated with differences in early childhood behavior. In particular, some evidence suggests children born via c-section may be at increased risk for delayed development of cognitive and motor behavior (8). Thus, we sought to investigate whether children born by c-section showed any similar deficits in feeding behaviors compared to vaginally born infants.

In the dataset analyzed, general appetite represented a single rating of infant appetite provided by mothers, food enjoyment was calculated from ratings related to whether infants liked eating, and satiety responsiveness was calculated from ratings related to how easily the infants got full (19).

We found that for up to 6 months of age, infants born via c-section trended towards higher general appetite, higher food enjoyment, and lower satiety-responsiveness than those born vaginally but largely did not show statistically significant differences (Fig. 1A-C).

The three trends we identified in our data have previously been shown to correlate with increased weight gain in infants (20, 21). Given the correlation of these scores with increased weight, we wanted to see if the c-section delivered infants in this dataset also trended towards increased weight. Mean weight for length z-scores of infants at each age sampled were calculated and plotted as a line graph. The resulting curves showed that c-section delivered infants indeed trended towards higher weight-for-length z-score than vaginally delivered infants (Fig. 1D). Interestingly, at the earliest time point, differences seemed to be minimal, but increased with time up until 6 months. At 9 and 12 months, the weight-for-length z-scores between the modes of delivery appear to converge. t-tests revealed that at all timepoints, the differences in weight-for-length z-score were not statistically significant. However, given the fact that the c-section delivered infants had greater weight-for-length z-score at all timepoints, we believe a trend may still exist within our data. Previous studies have found a similar increased risk for obesity among c-section delivered infants compared to vaginally delivered infants at 6 months of age, but not afterwards (22).

Overall infant gut microbiome did not differ between modes of delivery. Given the vast evidence supporting a difference in gut microbiome composition between c-section born and vaginally delivered infants, we sought to see if a similar difference in the microbiomes of c-section and vaginally delivered infants in the dataset we studied was present. The microbiome has previously been shown to have an influence on host appetite through production of

metabolites that affect satiety hormone release (23); if microbiome differences are seen, this may help explain the differences in feeding behavior we saw.

Data from microbiome samples were processed and beta diversity of samples between the modes of delivery were determined using weighted UniFrac analysis. The resulting Principal Coordinate Analysis (PCoA) plot showed overlap of points representing samples from c-section delivered infant microbiomes and those from vaginally delivered infant microbiomes, suggesting mode of delivery was not a major driver of microbiome variation (Fig. 2A).

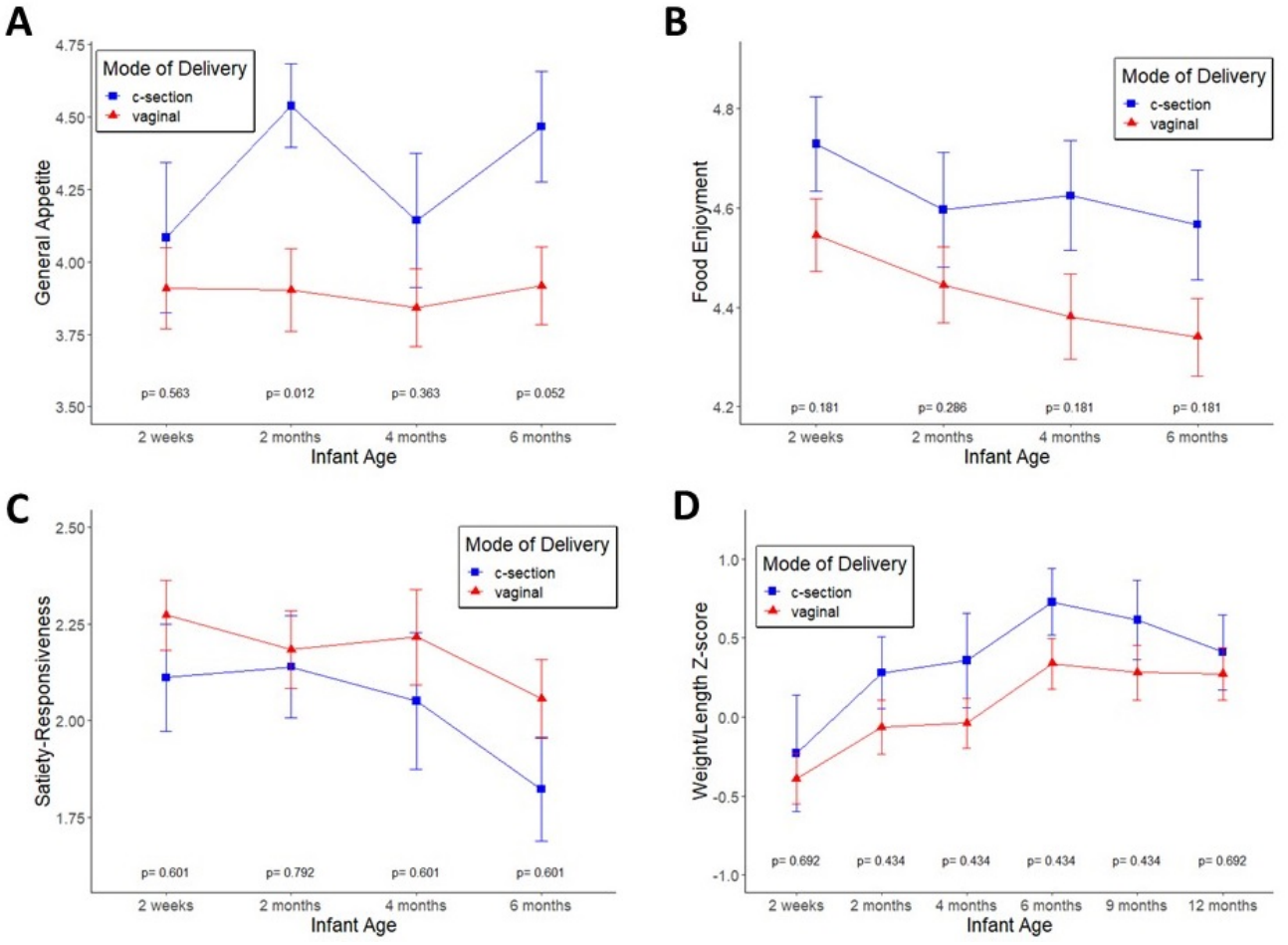

FIG. 1 Infants born via c-section show behavioral trends associated with increased weight gain and increased weight-for-length z-score compared to vaginally born infants. (A-C) For infants born either via c-section (blue) or vaginally (red), the mean scores of various feeding behaviors were calculated at 2 weeks, 2 months, 4 months or 6 months of age and plotted using R. (A) Mean General Appetite scores (In order of increasing age, for c-section: n = 12, 13, 14, 15, for vaginally born babies: n = 33, 41, 38, 36). (B) Mean Food Enjoyment scores (c-section: n = 12, 13, 14, 15, vaginal: n = 33, 41, 38, 36). (C) Mean Satiety Responsiveness scores (c-section: n = 12, 12, 13, 15, vaginal: n = 33, 40, 37, 35). (D) Mean weight-for-length z-scores of c-section (blue) or vaginally born (red) infants were calculated at 2 weeks, 2 months, 4 months, 6 months, 9 months or 12 months of age and plotted using R. (c-section: n = 13, 17, 17, 14, 12, 16, vaginal: n = 35, 42, 45, 39, 41, 39). P-values shown were calculated from two-sample t-tests in R and adjusted using Benjamini-Hochberg correction. Samples from infants of unknown mode of delivery were not included. Error bars represent standard error of the mean.

To investigate microbiome composition in more detail, we used Analysis of Composition of Microbiomes (ANCOM) to study differentially abundant genera between the modes of delivery (18). The resulting volcano plot revealed that only one genus was differentially abundant between the modes of delivery, the genus *Bacteroides* (Fig. S2). Relative abundance plots of *Bacteroides* in gut samples between c-section and vaginally delivered infants revealed that vaginally delivered infants had an increased relative abundance of *Bacteroides* compared to c-section delivered infants, where they were largely undetectable (Fig. 2B). This

is consistent with previous studies that have found colonization by *Bacteroides* species to be delayed or reduced following c-section birth compared to vaginal births (2).

Thus, while beta diversity and ANCOM analysis revealed that overall gut microbiome composition did not differ between the modes of delivery, relative abundance analysis revealed that at least *Bacteroides* levels differed. While *Bacteroides* species have been linked with regulation of host metabolism through metabolite production (24), the lack of overall differences suggest microbiome differences are a less likely explanation for the differences in feeding behavior and weight-for-length z-score seen earlier.

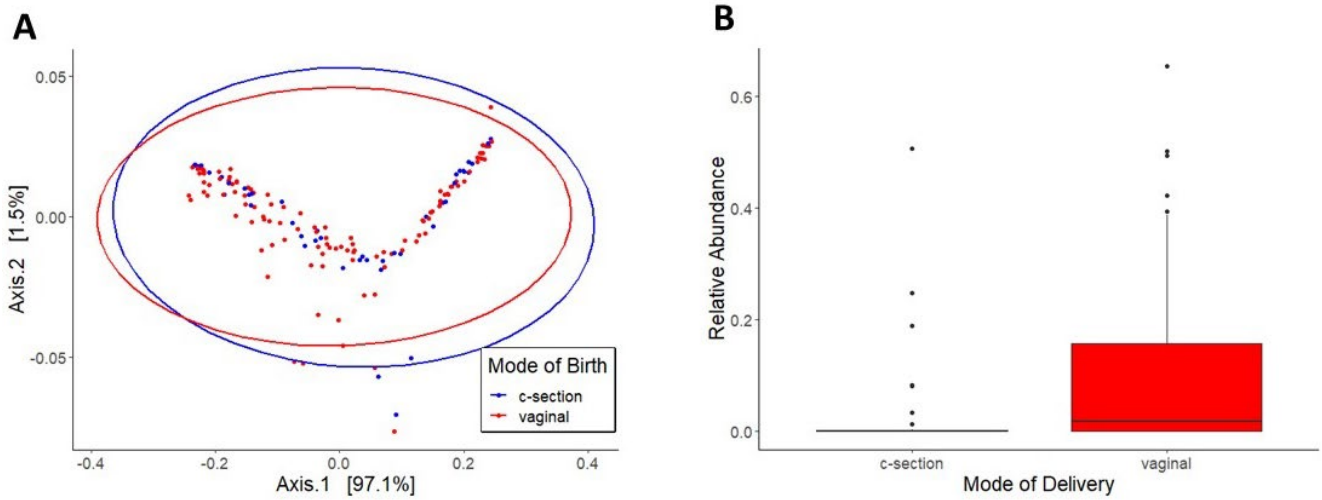

FIG. 2 Infants delivered via c-section or vaginally showed minimal gut microbiome differences except for Bacteroides abundance. (A) Variation between infant microbiome samples was visualized on a PCoA plot using Weighted UniFrac analysis in R. Samples from c-section delivered infants are represented in blue and samples from vaginally delivered infants are represented in red. Ellipses represent 95% confidence intervals. Samples from infants of unknown mode of delivery were removed prior to analysis. (B) Relative abundance of *Bacteroides* in gut samples between c-section and vaginally delivered infants. *Bacteroides* were determined to be differentially abundant using ANCOM analysis (w=195, Fig. S2). Boxes represent interquartile range, bolded lines within boxes represent median. Plots were created using ggplot in R.

Maternal BMI does not significantly influence infant weight-for-length ratios and satiety responsiveness. Higher maternal BMI has been associated with increased frequencies of c-section as the mode of delivery (25). Having studied the effects of mode of delivery on infant growth, feeding behaviour, and gut microbiomes, we next sought to study how maternal BMI can affect these infant characteristics.

We first investigated the effects of maternal BMI on infant weight-for-length ratios and satiety responsiveness. Previous studies have shown that males born to obese mothers had significantly increased body fat from ages 2-6 relative to those born to overweight and normal weight mothers (26). They further showed that there were no significant differences before age 2. Vaginally delivered infants are thought to inherit more of their mother's microbiome, which is important for immune system development and preventing metabolic diseases (6). We subsetted the data for vaginally delivered infants to enable downstream studies exploring the potential role of the gut microbiome in affecting weight-for-length z-scores. To confirm if the previously observed weight-gain pattern was cohort specific, we conducted a correlation study using longitudinal data and compared infant weight-for-length Z score between infants born to normal, overweight, and obese mothers over 12 months. We found that infants born to obese mothers had significantly higher weight-for-length compared to those born to overweight mothers ($p = 0.0107$) and normal mothers ($p = 0.00174$) at two weeks post-birth (Fig. 3A). However, there were no statistically significant differences after two weeks. There have been conflicting reports on maternal pre-pregnancy BMI and infant birth weight with some studies reporting increased maternal BMI being associated with low birth weights (27, 28) and others reporting associations between increased BMI, high birth weight for gestational age, and fetal macrosomia (abnormally large birth weight) (29–32). Although these reports focus on infant size for gestational age, we hypothesize our observation may be

due to obese and overweight mothers delivering babies with higher at-birth weights that persists 2 weeks in, thereby increasing the weight-for-length ratio. However, further studies such as collecting at birth weight information would be needed to better understand this statistically significant observation. Similarly, correlation between maternal weight category and infant satiety responsiveness showed no significant differences at any time point, suggesting little to no impact of maternal weight category on early infant feeding behaviour (Fig. 3B).

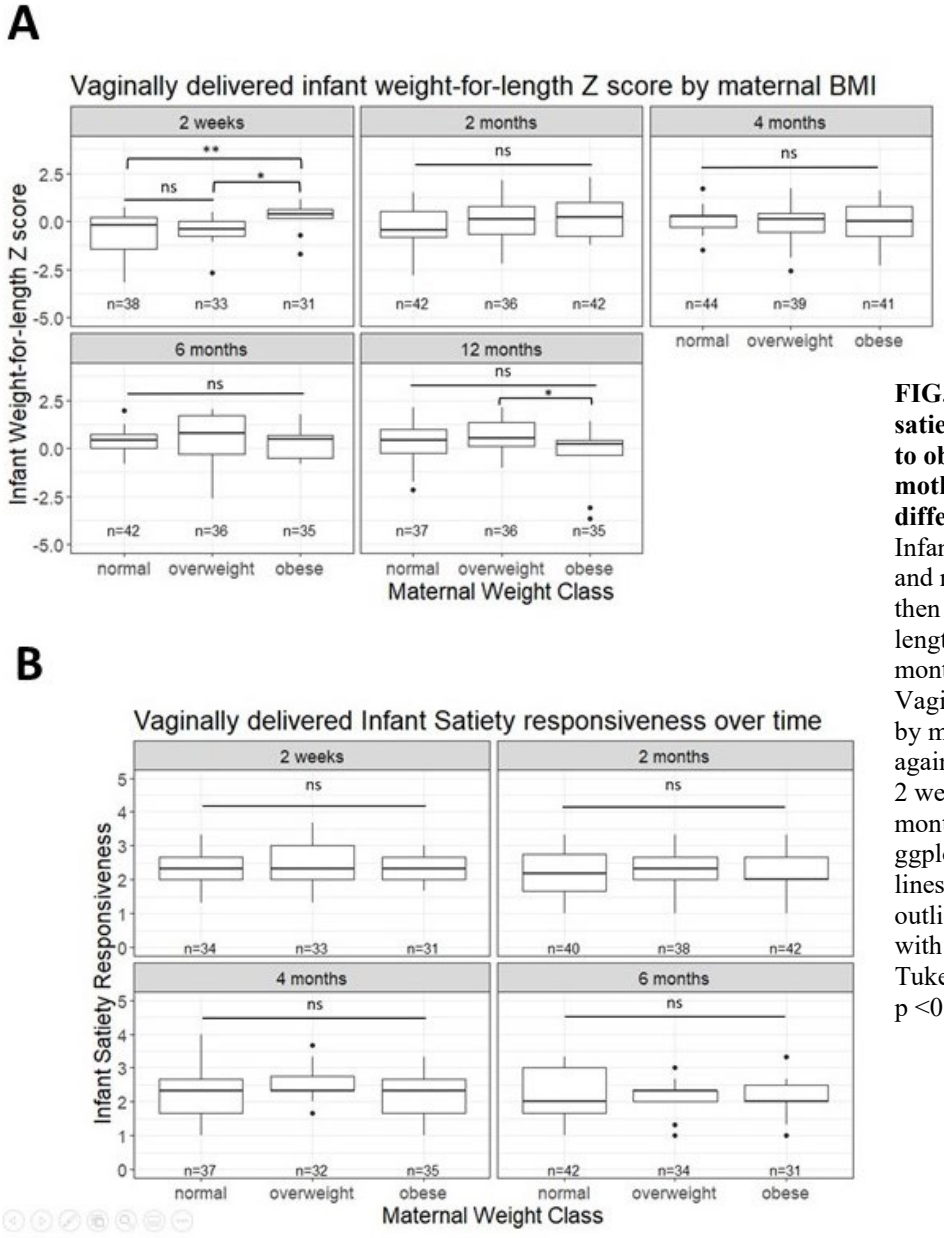

FIG. 3 Weight-for-length ratio and satiety responsiveness of babies born to obese, overweight, and normal mothers are not significantly different over 12 or 6 months. A) Infants were sorted by vaginal delivery and maternal BMI class. They were then plotted against infant weight-for-length Z scores at 2 weeks, 2 months, 4 months, 6 months, and 12 months B) Vaginally delivered infants were sorted by maternal BMI class and plotted against infant satiety responsiveness at 2 weeks, 2 months, 4 months, and 6 months. Figures were generated using ggplot in R. Boxes represent IQR, bold lines represent median. Points represent outliers. P-values were calculated using with pairwise comparisons using Tukey-Kramar tests (* = $p < 0.05$, ** = $p < 0.01$, ns = $p > 0.05$).

Maternal gut microbiomes are minimally different based on BMI classifications. Previous studies have shown differing gut microbiomes between obese and normal weight individuals (33) and the ability for gut microbiomes to be transferred during vaginal delivery (6). To determine if maternal microbiomes had significant differences based on weight class that could be passed on to infants, we analyzed maternal microbiome data using the weighted UniFrac quantitative measure of beta diversity and visualized the data using PCoA plots. Surprisingly, the PCoA plot for maternal microbiomes did not show distinct clustering between weight-classes and largely distributed equally along the Axis 1, which accounted for 92.5% of the diversity (Fig. 4A). This suggested that there are no large-scale microbiome differences in the mothers in this cohort. However, there was a relatively distinct cluster of

obese mothers in the bottom right-hand corner, suggesting there may be a subset of mothers with distinct microbiomes that could be further investigated. To further investigate the difference between maternal microbiomes, we conducted differential abundance analysis (DAA). We found that the *Paraprevotellaceae* family appeared to be differentially abundant (p.adjusted < 0.05). The role of *Paraprevotellaceae* in the human gut is not well studied in literature. Interestingly, the relative abundance plots did not appear to be significantly different (Fig. 4B), and we believe this may be due to a low relative abundance cutoff at > 0.0001 during DESEq2 analysis that captured very small differences. Therefore, there may be a possibility that the fold change may be very small and that this result could be a false positive.

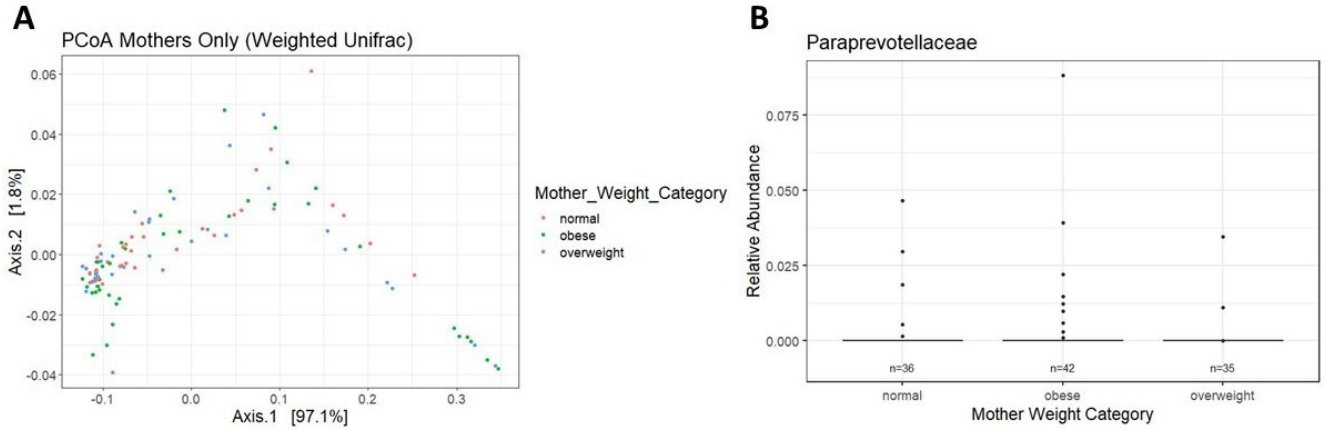

FIG. 4 Mothers of varying BMIs do not have significant differences in gut microbiome. A) PCoA visualization of differences in maternal gut microbiomes (Normal maternal weight = pink, Overweight maternal weight = blue Obese maternal weight = green). B) Relative abundance boxplot of *Paraprevotellaceae* family between normal, obese, and overweight mothers. Plots were created using ggplot in R. Statistical significance was determined using DESEq2 (p.adjusted < 0.05).

Infant gut microbiomes minimally differ by maternal BMI. While maternal microbiomes did not differ significantly by BMI, we looked to see if the same could be seen in infant gut microbiomes. As we observed a lack of significant differences in early life weight-for-length ratio and satiety responsiveness between infants born to mothers with different BMI classifications, we looked to investigate the microbial diversity in vaginally delivered infants to see if there were differences or similarities in composition that could support our earlier findings. Similar to maternal microbiomes, we analyzed the gut microbiomes of vaginally delivered infants using the weighted UniFrac measurement of beta diversity and visualized the results using a PCoA plot (Fig. 5A). Our results show there is no significant clustering by maternal weight class, suggesting that infant gut microbiomes do not significantly differ depending on their mother's BMI. It is interesting to note there was a small trend for infants with a normal BMI mother to occupy the left part of the PCoA plot, leaving a small cluster of obese and overweight infants on the right of Axis1. DAA using DESEq2 on vaginally delivered infants showed significant differences in the families *Erysipelotrichaceae* and *Xanthomonadaceae* (p.adjusted < 0.05). A previous article has shown that diet-induced obesity in animal models correlated with an increase in *Erysipelotrichaceae* (34). Furthermore, neonates born to overweight or obese mothers had stool with depleted *Xanthomonadaceae* (35). The relative abundance plot for *Erysipelotrichaceae* showed there may be a greater abundance of *Erysipelotrichaceae* in infants born from obese/overweight mothers compared to infants born to normal mothers (Fig. 5B). However, the relative abundance plot for the *Xanthomonadaceae* family showed little differences between the boxplots, suggesting that the low relative abundance cutoff at > 0.0001 during DESEq2 analysis may have captured very small changes that may not be biologically significant (Fig. 5C). Together, these data suggest that vaginally delivered infant microbiomes differ slightly based on maternal BMI classifications with perhaps *Erysipelotrichaceae* having a biologically significant difference in abundance.

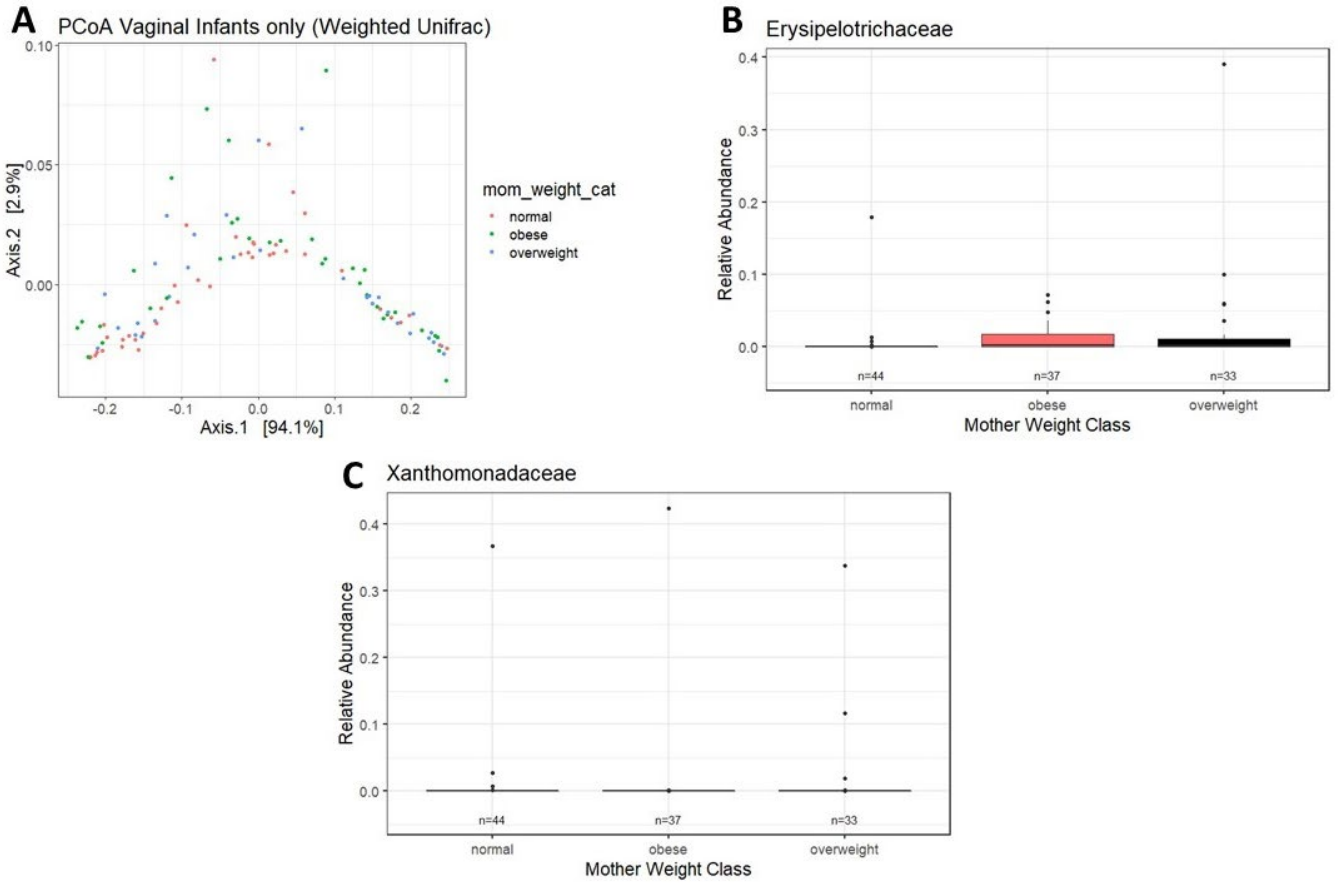

FIG. 5 Gut microbiome is not significantly different between infants born to mothers of varying BMIs. A) PCoA visualization of differences in vaginally delivered infant gut microbiomes. Beta diversity calculated using weighted UniFrac (Normal maternal weight = pink, Overweight maternal weight = blue, Obese maternal weight = green). B) Relative abundance boxplot of gut *Erysipelotrichaceae* between infants with normal, obese, and overweight mothers C) Relative abundance boxplot of gut *Xanthomonadaceae* between infants with normal, obese, and overweight mothers. Plots were created using ggplot in R. Statistical significance was determined using DESEq2 (p.adjusted < 0.05).

DISCUSSION

The gut microbiome has been shown to have an intricate relationship with obesity (33, 36). Recent studies have linked pre-pregnancy maternal obesity with increased prevalence of child obesity (37), so studying the effects of maternal BMI on infant gut microbiome and early life development may elucidate areas for early intervention to prevent childhood obesity.

Previous studies have reported differences in early life behavior between c-section and vaginally delivered infants, with c-section delivered infants often showing delayed development (8). Here, we showed that differences may exist in feeding related behaviors between infants born by c-section and vaginally, with c-section born infants trending towards increased general appetite, increased food enjoyment and decreased satiety-responsiveness. These trends have previously been associated with increased weight gain (20, 21). Thus, the behavioral trends we see in our data may provide a potential mechanism for why c-section delivered infants have been shown to have an increased risk for weight gain compared to vaginal infants (22, 38). Indeed, we found that c-section delivered infants trended towards higher mean weight-for-length z-score than vaginally delivered infants at all timepoints in the dataset we studied. Interestingly, these differences decreased with age, a phenomenon also previously described (22, 38).

In addition to investigating feeding behavior between the modes of delivery, we also investigated microbiome differences between the modes of delivery. We found minimal microbiome differences between the modes of delivery, with almost complete overlap of clusters on PCoA plots. Contrary to our findings, a number of studies have reported clear microbiome differences between vaginally and c-section delivered infants, with several taxa

being differentially abundant. However, across these findings, *Bacteroides* has been consistently identified as being differentially increased in abundance in vaginal compared to c-section births (6, 39). This was indeed consistent with relative abundance analysis we performed, which found increased relative abundance of *Bacteroidies* in vaginally compared to c-section delivered infants. Thus, while our findings are not entirely consistent with previously described trends, they do lend support to an identified trend. This is of note as *Bacteroides* have been identified as a genus containing key commensal species, with roles in host metabolism and opportunistic pathogen resistance (24).

The other potential early life determinant we explored was maternal BMI. Looking into the relationship between maternal BMI and infant early life development, we found significant differences in infant weight-for-length Z score at 2 weeks, but none after 2 weeks. This finding agrees with a previous finding that children born to obese mothers did not show body fat differences until age 2 (26). We hypothesize that the significant differences in infant weight-for-length Z score observed in week 2 may be due to higher BMI mothers delivering infants with higher birth weights as shown in a previous study by Shapiro et al. (40). Similar to weight-for-length, correlating infant satiety responsiveness with maternal BMI did not show any significant differences, together suggesting maternal BMI did not significantly influence early infant weight-for-length and feeding behaviour.

We then investigated if there are distinct differences in gut microbiomes among mothers classified as normal, overweight, or obese. We showed that the maternal microbiomes did not form largely distinct clusters based on weight status for a weighted Unifrac PCoA plot. However, we did observe an interesting cluster of several overweight and obese mothers that were separated from the remaining mothers, suggesting amongst mothers from the same state and country (Michigan, United States), there may be a group of individuals with microbiomes distinct from the larger population associated with greater BMI. Future work can be done to identify these mothers by their anonymized names and compare these individual's microbiome composition to those of individuals outside this cluster using DESeq2.

Furthermore, while DAA identified the family of *Paraprevotellaceae* to be differentially abundant between mothers of different BMI classifications, relative abundance boxplots showed that the differences were extremely small and perhaps not biologically significant. These results were somewhat surprising as we expected more distinct clustering based on weight classes as previous literature have linked certain microbiome compositions with obesity (41, 42). We believe factors such as identical country of birth (United States) and state of residence (Michigan) may be possible causes of the similar microbiotas we observed.

While our data suggested there was little gut microbiome difference by weight class in mothers, we looked to see if the same could be seen in infants to observe if mothers selectively pass on certain beneficial or aberrant bacteria to their offspring. Expectedly, we did not observe significant differences in infant gut microbiomes categorized by maternal weight category, which is consistent with our previous finding of little difference among the mothers. Interestingly, we saw a different set of differentially abundant bacteria in infants compared to their mothers. We investigated two of these families of bacteria, *Erysipelotrichaceae* and *Xanthomonadaceae*, and found that relative abundance boxplots for *Erysipelotrichaceae* showed increase abundance in infants born to obese/overweight mothers while little to no differences could be seen in the boxplots for *Xanthomonadaceae*. This suggests differences in *Erysipelotrichaceae* are biologically significant while *Xanthomonadaceae* differences may be a false positive. Previous studies have shown that *Erysipelotrichaceae* are associated with metabolic disorders in animal models and human correlational analyses (43–45). This may suggest infants who are born to obese/overweight mothers and have increased *Erysipelotrichaceae* may have an increased risk of weight gain or other metabolic diseases. However, further studies will be required to both identify the role of *Erysipelotrichaceae* in humans and in developing obesity.

Our results showed that infants have different abundances of microbiota compared to mothers at least in the early stages of their lives. It would be important to note that it is still likely that mothers pass on their gut microbiota to their offspring in accordance with a previous study that found the maternal gut microbiota to be the major source of transmitted organisms (46). However, our data suggests that early life factors may alter both composition and relative abundance in infants compared to their mothers. We believe environmental

factors including hospitals, homes, and diet may play significant roles in our observations. Future studies that isolate infant-mother dyads can help understand how the microbiome composition and relative abundance compares between mother and child.

Limitations A major limitation in this study was the relative abundance threshold in the DAA using DESEq2. We tested different thresholds, but found that thresholds greater than 0.0001 produced an error. We think this may be due to there being no relative abundance differences that are greater than 0.0001. However, further analysis with relative abundance boxplots suggested that the threshold may have been set too low, and as a result, DESEq2 analysis captured miniscule differences that were later determined as statistically significant despite them not being biologically significant.

Another limitation was that all infants and mothers in the dataset we used were born in Michigan, United States. This introduces bias in our observations: the various child-mother pairs may experience similar life conditions, and as a result, develop more similar gut microbiota as other pairs. Further, this limited dataset restricts the possible extrapolations we could make to people in the Midwestern United States. Another limitation is that when attempting to analyze the effects of mode of delivery, we were not able to control for potential confounding factors. Of note, differences in feeding method and early antibiotic exposure have been linked with differences in infant behavior, weight gain and microbiome composition in human and murine studies (47–50). Controlling for these factors may yield more distinct differences, but it was not done in this study due to a low sample number in the resulting subsets. Future analysis using linear regression models may allow for controlling of these factors without reducing sample size. Another potential confounding factor for the microbiome analysis may have been time, as microbiome differences between modes of delivery have been shown to decrease with age (39). Controlling for time during the beta diversity and relative abundance analyses may have led to more distinct differences. Finally, the dataset we studied used 5 point surveys to quantify feeding behavior scores. In addition to the inherent subjective nature of surveys, the use of a 5 point system as opposed to a larger point system may have prevented smaller behavioral differences from being captured, potentially causing subjects to artificially rated as similar.

Conclusions Our study investigated two potential determinants of the infant gut microbiome and early life development: baby delivery method and maternal BMI. Through correlational studies and microbiome analysis, we determined that these potential determinants did not have significant impacts on the overall infant gut microbiome and early life development in this cohort. However, taxonomic analysis revealed increased differential abundance of the genus *Bacteroides* in vaginally delivered infants, and the families *Erysipelotrichaceae* and *Xanthomonadaceae* in infants born to obese/overweight mothers. Together, the findings indicate that delivery modes and maternal BMI can potentially lead to small-scale changes to the microbiota, identifying areas for further research on early infant health.

Future Directions In future studies, it would be important to enforce stricter testing conditions where variables that are known to impact infant gut microbiota and early life behavior will be controlled for. For example, infants with antibiotic usage or prematurely born infants should be subsetted before any downstream analysis, or linear regression analysis could be done to control for these (51, 52). Further, analysis of metadata categories should be subset by time to control for infant age. While this was done in the present study for the feeding behavior analysis, doing so for other analyses led to too low of a sample size.

If there is enough funding, a similar but larger clinical study should be performed to account for individuals who might withdraw or who do not provide data for certain questions. In addition, a larger sample size would allow researchers to subset the data and still retain enough samples to determine statistical significance. Similarly, a study looking at different populations, both geographically and/or culturally, may yield data that could be extrapolated to the general public with more certainty.

In our study, we demonstrated that mode of delivery was correlated with differences in feeding behavior, as well as differential abundance of *Bacteroides* bacteria in gut microbiome samples. Seeing as *Bacteroides* species have been demonstrated to have a role in nutrition

and metabolism in adults (24), a future study could investigate if *Bacteroides* abundance is a potential driver for these behavioral differences in infants. For example, a correlational analysis on a larger scale or experiments on murine models may be done.

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CONTRIBUTIONS

All authors contributed equally to the writing and editing of the report, to conception of project ideas, and analysis and discussion of results. E.Y. and L.L. were responsible for data analysis and presentation regarding mode of delivery. E.T. and L.L. were responsible for the data analysis and presentation regarding maternal BMI.

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