Volume 8:1-11

UJEMI+

The Undergraduate Journal of Experimental Microbiology & Immunology (+Peer Reviewed)

Infant gut microbiota diversity and composition as a potential link between feeding method and atopic dermatitis

Melanie Law, Irene Jiang, Jing Wen, Karen Xie

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY **Background:** Atopic dermatitis (AD) is a chronic inflammatory skin disease with no known cause or cure, often beginning in the first year of life. The gut microbiota, which is proposed to play a role in AD development, is integral in immune system development and regulation. Breastfeeding shapes the gut microbiota and has been observed to lower AD incidence. Thus, it has been viewed as a type of early intervention to prevent AD. Here, we investigate the microbiota as a link between feeding method and AD to contribute to ongoing research on this topic, which may inform future infant diet recommendations.

Methods: Stool samples from 325 mother-infant dyads at various time points over a 1 year period, collected by Dr. Kyung Rhee at the University of California San Diego, were analyzed for their microbial composition. A Pearson's chi-square test was performed to test for correlation between feeding method (breastfeeding and formula feeding) and AD status in infant samples. Alpha and beta diversity analysis and taxonomic classification were then conducted in QIIME2. Infant samples were stratified by feeding method, then differential abundance and relative abundance analysis were conducted using DESeq2.

Results: We report a significant correlation between feeding method and AD status, with higher AD incidence observed in the formula-fed group. We found that breastfed infants had greater Faith's phylogenetic diversity (PD) compared to formula-fed infants. However, there was no difference in alpha diversity based on infant AD status. Furthermore, breastfeeding was found to only favour the abundance of certain genera in healthy infants. In comparison, formula feeding favours the abundance of different genera in both healthy infants and AD infants.

Conclusion: Our results support our hypothesis that infant feeding method influences the development of AD by altering gut microbial diversity and structure. The present study validates previous literature that breastfeeding has protective effects against AD development. Furthermore, our work provides evidence that this relationship may be linked through the gut microbiota.

Published Online: September 2022

Citation: Law M, Jiang I, Wen J, Xie K. 2022. Infant gut microbiota diversity and composition as a potential link between feeding method and atopic dermatitis. UJEMI+ 8:1-11

Editor: Gara Dexter, University of British Columbia

Address correspondence to: Melanie Law, melanielaw72@gmail.com

INTRODUCTION

Atopic dermatitis (AD), commonly known as eczema, is a chronic inflammatory skin disease characterized by dry, itchy, and cracked skin which can vary in severity (1). These symptoms can range in the level of pain experienced and often have implications on the self-esteem levels in children and youth (2). As a chronic inflammatory condition, it is characterized by dysregulation of immune responses, resulting in altered cytokine production both systemically and within skin lesions (3). It is estimated that 15% to 20% of children worldwide are affected by AD, and 90% of children affected by AD develop the disease by

age 5 (4). Although the exact cause of AD has yet to be elucidated, it has been proposed that both genetics and environmental factors play a role in increasing the risk of AD development (5, 6).

There is a strong correlation between the modulation of the gut microbiome and the development of abnormal host immune behaviour (7). Perturbations in the early gut microbiota have been linked to an increased disease risk and dysbiosis (8). For example, decreased diversity in the gut microbiome of infants has been associated with the occurrence of some diseases, including AD and asthma (9). The gut microbiota plays an important role in infant AD development as it regulates the maturation of the immune system through microbiome and host cross-talk in early life (10). As such, early intervention during infancy is being viewed as a window of opportunity to modulate the gut microbiome in hopes of preventing the development of allergic manifestations, such as AD.

Diet content, in terms of both the quantity consumed and the timing of consumption, were shown to impact host immune function by modulating the composition of the host microbiome (11). In particular, breastfeeding is suggested as a potential intervention during infancy due to its effects on the infant's early gut microbiota. Cioffi *et al*. has shown a decrease in phylogenetic diversity and difference in gut microbiota composition in breastfed infants (12). Oligosaccharides, maternal IgA, and antimicrobial factors contained in human breast milk influence neonate gut colonization (8).

There have been numerous studies which observed that breastfed infants had a lower incidence of AD (11–16). Links between the gut microbiota, breastfeeding, and AD onset were proposed in a previous study by Wopeiris *et al*. exploring the effects of breastfeeding and interventions on colonization patterns in fecal microbiota (17). Work by Lee *et al*. found that the infant gut microbiota differed depending on infant feeding type and found a significant relationship between the reduction of mucin-degrading bacteria and immune development, indicating that associations between AD and gut microbiome modulation are due to bacterial genes that impact immune cell functionality in the host (18).

Specific bacterial taxa have been found to be associated with AD onset. For example, the genera Parabacteroides and *Clostridium sensu stricto* are found to be more abundant in AD patients, where Enterococcus are associated with decreased incidence of AD (19–21). Specifically, *Clostridioides difficile* (formerly *Clostridium difficile*) is reported to have a direct effect on cytokine production and inflammatory responses (22). The presence of C. difficile leads to a decrease in other beneficial bacteria, resulting in suboptimal levels of immune regulation (23). Furthermore, toxins A and B produced by C. difficile increase the intestinal permeability and facilitate the penetration of innocuous antigens, and subsequent atopic sensitization development (24).

In this paper, we investigate the microbiota as a link between feeding method and AD using a dataset generated by Dr. Kyung Rhee from the University of California San Diego. This dataset was collected from 325 infant-mother dyads over a period of 1 year with the original purpose of investigating the relationship of infant gut microbiota and fecal metabolomics to infant weight gain and eating behaviour. The dataset is available on the European Nucleotide Archive (ENA) browser (PRJEB39437).

We hypothesize that the feeding method influences the development of AD through the infant gut microbiota by altering its microbial diversity and structure. As feeding method influences the gut microbiota and the microbiota is integral in immune system development and regulation, alterations can significantly impact the immune environment, leading to inflammatory diseases like AD. Investigating this link may provide insights to clinical recommendations regarding the infant diet.

METHODS AND MATERIALS

Dataset. This project utilizes a dataset generated by Dr. Kyung Rhee from the University of California San Diego, which was generated by examining the microbial composition of stool samples originating from 325 infant-mother dyads over the course of 12 months. This data was collected to elucidate how the gut microbiome is associated with infant eating behavior and weight gain within the aforementioned time frame, as well as to identify intervention targets for abnormal weight gain. Additional factors impacting infant microbiome development such as the mother's microbiome and infant medical history were also included.

This dataset is available through the European Nucleotide Archive (ENA) browser (PRJEB39437) and contains 11,221,040 reads across 307 samples, with reads ranging up to 150 nts (98% of reads) in length.

Feeding method and AD association. To assess if there was a significant relationship between AD development and feeding method, a Pearson's chi-squared test was performed using R (V.4.1.1) (25). The accompanying metadata file was used to carry out this assessment.

Preprocessing and diversity analysis. Demultiplexed single-end sequence data were imported into Quantitative Insights into Microbial Ecology Version 2 (QIIME2) through the manifest format approach and denoised using DADA2 (26, 27). Reads were truncated at 129 bp to maintain an average Phred score of 38 (Figure S1). To account for unequal sample sequencing depth, samples were rarified at a depth of 26,745 sequences per sample to capture sufficient ASV saturation (Figure S2). Metrics for alpha and beta diversity were calculated and visualized through QIIME2. These included Shannon's diversity index, observed features, Faith's phylogenetic diversity (Faith's PD), and Pielou's evenness for alpha diversity (28, 29). Beta diversity was assessed through Jaccard distance, Bray-Curtis distance, unweighted, and weighted Unifrac (30-32). Fragment insertion phylogenetic trees were generated using SILVA 128 SEPP as the reference (33). ASV taxonomic classification was assigned using a pre-trained classifier (silva-138-990515-806) available through QIIME2. To assess if the time of sample collection was confounded with weighted Unifrac, Principal Coordinate Analysis (PCoA) plots were constructed for cluster identification.

Taxon abundance. QIIME2 artifacts for ASVs with assigned taxonomy, metadata, and phylogenetic tree were combined and converted into a phyloseq object using R (V 4.1.1) with CRAN packages tidyverse, vegan, and Bioconductor package phyloseq (34–36). Samples with a sequencing depth of fewer than 10,000 reads were removed. The remaining samples were stratified by feed type (breastfed and formula-fed) and all samples where AD status and feed type were not reported were removed. Relative abundance was calculated to normalize taxon abundance across samples (37). Only features with a relative abundance greater than 0.005% were retained and analyzed at the genus level. Analyses for specific species of interest, such as Clostridiodes diffcile were performed at the species level. Differential abundance analysis between healthy and AD infants within each feed type was performed in R using DESeq2 (38). Relative abundance levels of taxa in healthy infants were used as the reference in comparison to those with AD. The threshold for significance was set to an alpha value of 0.05.

RESULTS

Age at sample collection did not influence gut microbial beta diversity

To determine if all time points should be included in our analysis, we generated a weighted UniFrac PCoA plot based on sample collection time (Figure 1). No clear clustering

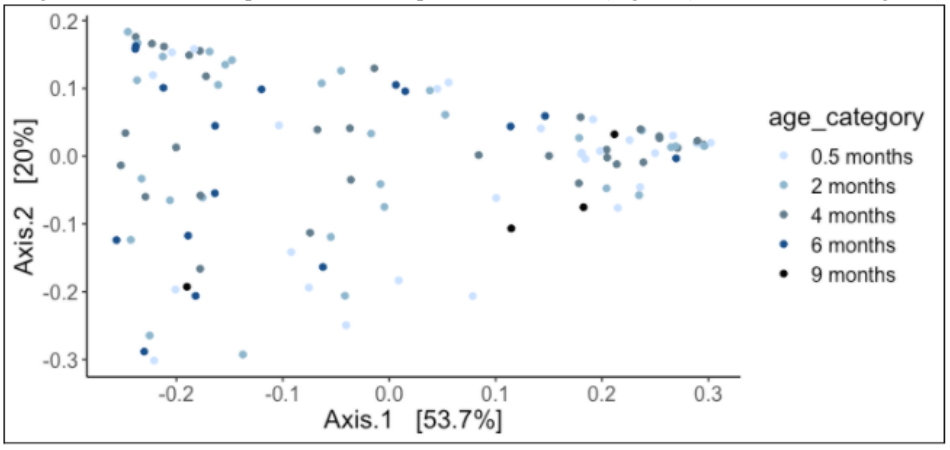

FIG. 1 Time of sample collection did not impact beta diversity of infant gut microbiotas. Weighted UniFrac PCoA plot based on time of sample collection showed no distinct clustering.

based on time of collection was observed, which indicates that the time of collection/infants' age did not impact beta diversity of infant gut microbiota. As such, time was not considered a confounding variable for our analysis. All of the time points from 2 weeks to 9 months were included.

Feeding method was correlated with AD onset

The relationship between feeding method and AD onset was first explored to determine if there were notable results warranting further investigation. We found a significant correlation between feeding method and AD as revealed by Pearson's chi-square test, in which a higher percentage of AD was observed in formula-fed infants (Figure 2).

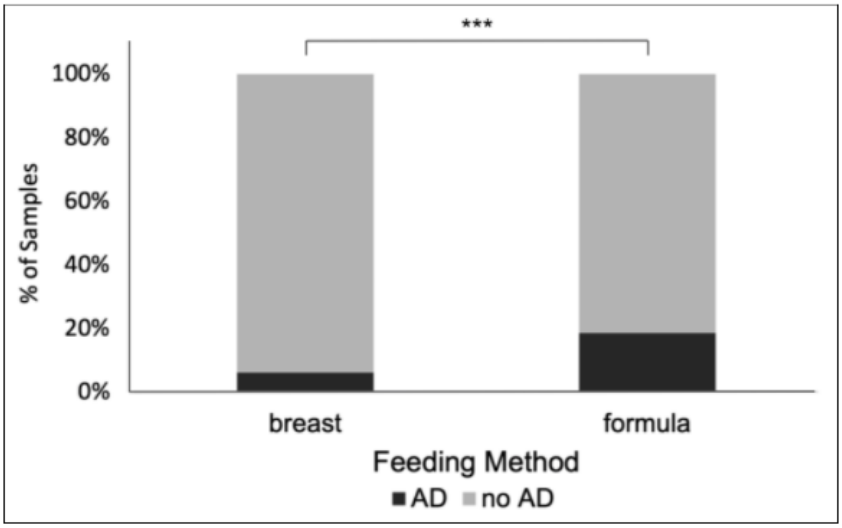

FIG. 2 Feeding method was significantly correlated to AD status. Stacked bar chart shows a higher percentage of AD infants within the formula-fed group. Pearson's chi-square test reports a significant correlation between feeding method and infant AD status (*p-value = 0.01).

Feeding method had a significant effect on alpha diversity but AD status did not impact the alpha and beta diversity of the infant gut microbiota

To determine if infant gut microbial diversity and composition varied between different feeding methods and their AD status, we performed Faith's PD alpha diversity and weighted UniFrac distance beta diversity analysis.

Alpha diversity analysis revealed that infant gut microbiotas did not change significantly between healthy and AD infants, as determined by a Kruskal-Wallis test with p-value greater than 0.05. This indicated that the microbial diversity was not affected by infant AD status (Figure 3A). The weighted UniFrac distance PCoA generated for beta diversity analysis based on AD status demonstrated no distinct clustering, suggesting that the microbial composition of samples differed from one another regardless if the infant was healthy or had AD (Figure 3C). Therefore, AD status did not impact gut microbial diversity and structure in infants.

In terms of infant feeding type, it was found that formula-fed infants had a significantly higher Faith's PD than breast-fed infants (Figure 3B). The lack of clustering in the weighted UniFrac distance PCoA plot by feeding type suggested that the microbial structure of individual samples differed from one another, regardless of whether the infant was breast or formula fed (Figure 3D). This was further confirmed by a PERMANOVA significance test ($p > 0.05$). Collectively, this suggested that feeding type had a significant impact on infant gut microbial alpha diversity but not microbial composition.

Breastfeeding favoured four genera in healthy infants while formula feeding favoured different genera depending on AD status

Differential and relative abundance of genera between healthy and AD infants within breastfed and formula-fed infants were explored. Relative abundance analysis on breastfed infants revealed four genera exhibiting a higher abundance in healthy infants, which included *Erysipelatoclostridium, Enterococcus, Parabacteroides,* and *Clostridium sensu stricto* (Figure 4A - D). Among these four genera, *Clostridium sensu stricto* represented the highest $\log_2$ fold change.

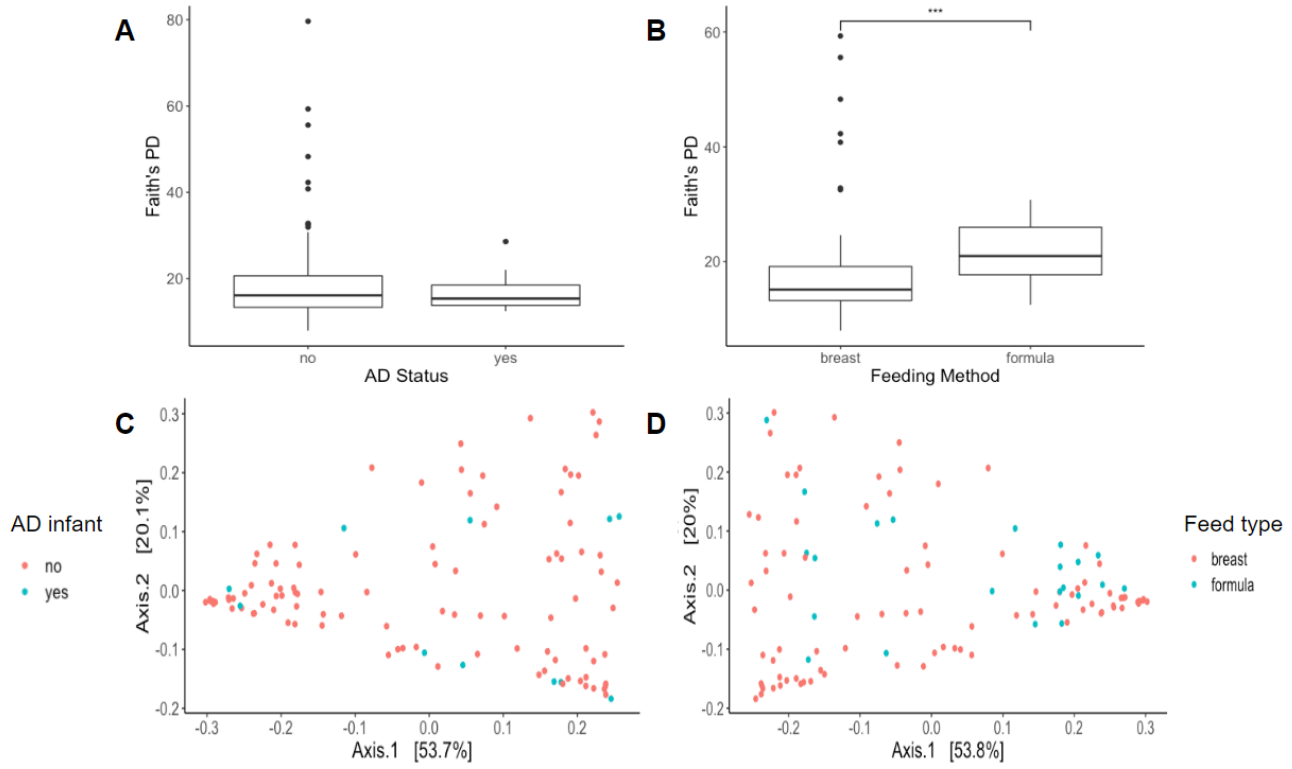

FIG. 3 Feeding method, but not AD status, was associated with differences in gut alpha-diversity, and neither were associated with differences in gut beta-diversity. Boxplots show that A) infants with AD have insignificantly lower Faith's PD than healthy controls, with a Kruskal-Wallis test p-value of 0.7, and B) formula fed infants have significantly higher Faith's PD than breastfed infants, with a Kruskal-Wallis test *p-value of 0.0003. Box plots show the interquartile range (25th, 50th, 75th percentile), whiskers represent the maximum and minimum, puncta represent outliers. Weighted UniFrac Distance PCoA plots based on C) AD status and D) feeding method showed no distinct clustering, suggesting neither significantly impact gut beta-diversity.

Through relative abundance analysis on formula-fed infants, it was found that at the genus level, AD infants possessed a significantly higher abundance of *Candidatus Stoquefichus,* while non-AD infants possessed a significantly higher abundance of *Faecaitalea* and *Acinetobacter* (Figure 4E - G).

Abundance of *Clostridiodes difficile* did not differ based on AD status

Previous literature suggests that *C. difficile* plays an important role in AD development in infants, by modulating the gut microbiota leading to subsequent sensitization development (23, 24). As such, we investigated the relationship between feeding method and levels of *C. difficile,* by calculating its relative abundance in formula-fed and breastfed infants. Our analysis found no significant differences in the relative abundance of *C. difficile* between healthy and AD infants, regardless of the feeding method (Figure 5A and B). This indicated that the feeding method and infant AD status did not influence the relative abundance of *C. difficile.*

DISCUSSION

Given known associations between infant feeding methods and the development of AD (17, 38), the objective of this study was to investigate the gut microbiota as a link between the two. Our results support the hypothesis that feeding method alters the diversity and structure of an infant's gut microbiota, which in turn likely introduces aberrations in immune system development and regulation, leading to an altered immune environment that favors the establishment and progression of inflammatory diseases such as AD. A significant

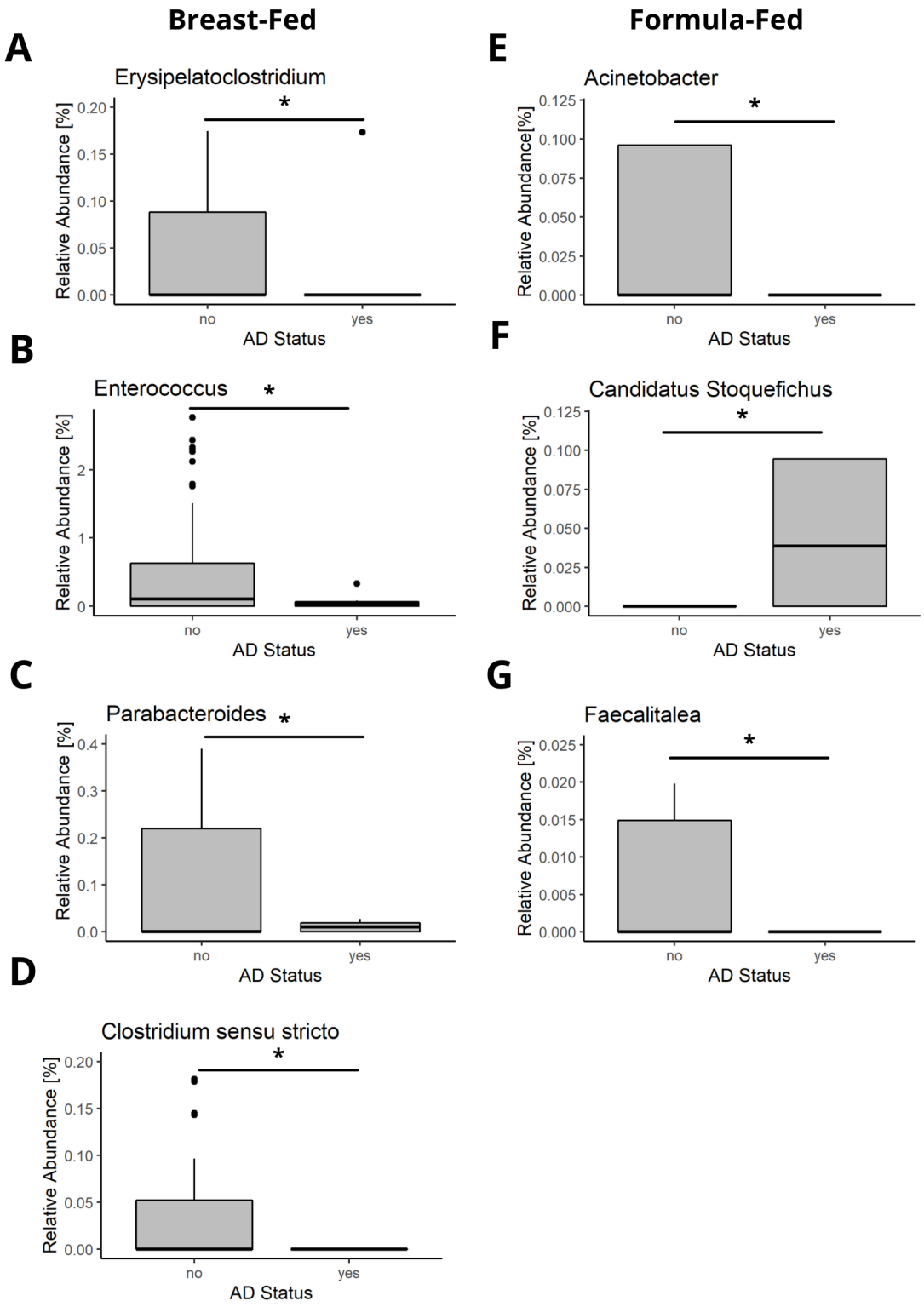

FIG. 4 Breastfeeding favours four genera in non-AD infants while Formula feeding favour different genera in AD and non-AD infants, revealed by relative abundance analysis. Relative analysis on formula-fed infants highlighted four significantly abundant genera in non-AD infants: (A) *Erysipelatoclostridium*, (B) *Enterococcus*, (C) *Parabacteroides*, and (D) *Clostridium sensu stricto* genus. Relative analysis on breastfed infants revealed a higher abundance of (E) *Acinetobacter* in non-AD infants, and a higher abundance of (F) *Candidatus Stoquefichus*, and (G) *Faecalitatea* in AD infants. All differences were reported to be significant, determined with DESeq2 (*p.adjusted <0.05).

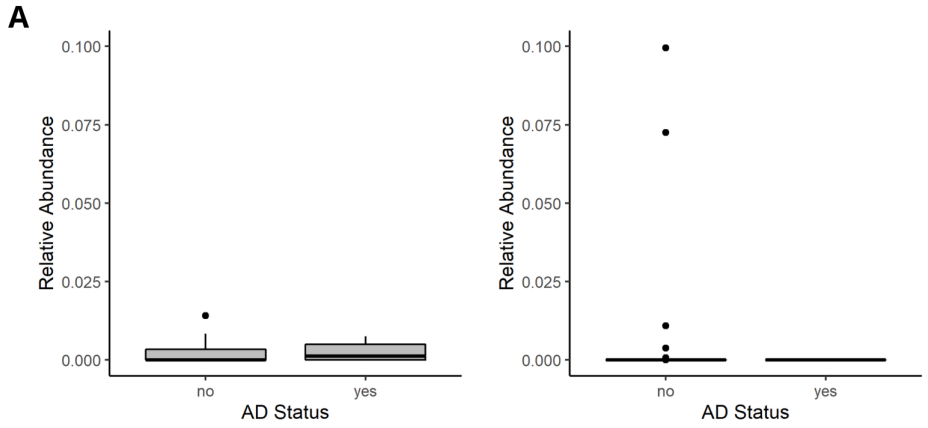

FIG. 5 No significant difference in relative abundance of *Clostridiodes difficile* between AD and non-AD infants. Relative abundance of *Clostridiodes difficile* was performed on (A) formula-fed infants and (B) breastfed infants. Differences were reported to be not significant, as determined by DESeq2 (*p.adjusted > 0.05).

correlation found between feeding method and AD supports current literature that describes heavily integrated roles of the gut microbiota in both.

Our study validates previous findings that breastfeeding has a protective effect against the development of AD, as breastfeeding was associated with a lower prevalence of the condition (38). We also showed that breastfed infants had lower gut diversity compared to their formula-fed counterparts. Published literature on the early gut microbiota showed that species, such as Bifidobacterium, dominate in microbiota of breastfed infants. These species were found to limit the growth of other potentially pathogenic species (9). In our study, relative abundance analysis was performed on infant gut microbiota with different AD statuses within breastfed or formula-fed infants. This method differs from Ma et al.'s study which directly compares gut microbiota of infants with different feeding methods. As a result, Bifidobacterium did not show up in our present analysis. However, the presence of Bifidobacterium may still be a contributing factor of the lower observed alpha diversity in breastfed infants in this study. Conversely, formula-fed infants develop a more diverse microbiota early on, with genus *Enterobacteria, Clostridia, Enterococci*, and *Bacteroides* suggested from differential abundance analysis (39, 40).

Due to the nature of this study, we were unable to draw mechanistic conclusions. However, differences in the way breastfeeding versus formula feeding impacted the taxonomic profile of the gut microbiota in AD and non-AD infants suggest that alterations in the gut microbiota of AD infants may be masking the impact of feeding type on the microbiota. Whether this is due to the presence of specific taxonomic groups or the effects of interactions between certain clusters remains unclear. Nonetheless, the increased abundance of *Erysipelatoclostridium*, *Enterococcus*, *Parabacteroides*, and *Clostridium sensu stricto* observed in the breastfed non-AD group, the increased abundance of *Faecalitalea* and Acinetobacter in the formula-fed non-AD group, and the increased abundance of *Candidatus stoquefichus* in the formula-fed AD group may provide insight into how atopic dermatitis associated gut alterations respond differently to feeding methods than healthy gut microbiotas. Understanding the functional roles of and interactions between these differentially abundant taxa is a critical next step.

Relating to existing literature, *Erysipelatoclostridium* has been shown to contribute to the dysregulated immune environment observed in Crohn's disease patients, although research on its role in AD is lacking (41). It is reasonable to hypothesize that *Erysipelatoclostridium* contributes similarly to the immune dysregulation associated with AD development, characterized by a more hyper-inflammatory environment (42). In line with our present findings, the literature shows that the presence of Enterococcus in the gut reduces the risk of AD development in infants, although further causative studies are needed (21, 43). On the other hand, our findings contradict Reddel *et al*. and Marrs *et al.*'s findings of increased abundance of *Paracteroides* and *Clostridium sensu stricto* within the AD group (19). This discrepancy may be due to our limited sample size, or other factors such as composition of formulas and differences in study population. Furthermore, the increased presence of Acinetobacter in non-AD infants may play a role in immune regulation as they are associated

with the expression of anti-inflammatory molecules in healthy individuals by stimulating IL-10 production and inhibiting Th1 polarization (44). Therefore, lower abundance in the AD infants may be contributing to suboptimal levels of immune regulation, leading to AD development. In addition, a higher abundance of C. difficile was observed amongst the formula-fed group, a statistically insignificant finding that nonetheless supports previous research showing *C. difficile's* role in sensitization development (23, 24).

Limitations Our study is limited by several factors, including potential confounding variables, small sample size, ambiguity in data collection, inherent limitations of 16S rRNA amplicon sequencing, and limitations of QIIME2 pipeline.

All samples included in this study were derived from infants who were born in the United States and residing in Michigan. As such, similarities in their gut microbiota may be a result from exposure to similar environmental factors, which is a potential confounding factor. Therefore, insights drawn from this study may not be representative of the impact of feeding method on the development of the infant gut microbiota in other geographical locations.

Additionally, it is worthy to note that the time point of AD diagnosis was not consistent across all infants. Some infants received their AD diagnoses at a later time point than others. As such, samples from infants before and after they were diagnosed with AD were included in both AD and non-AD presenting groups, which may have impacted the dynamic range of observable differences between these groups. Furthermore, not all time points were collected for each sample to sufficiently track gut microbiome changes before and after AD diagnosis.

Our study is also limited by the small sample size; after filtering the samples collected from the mother, and excluding samples for which feeding method and/or AD status was not provided we had 296 samples in total and only 26 AD samples, which may contribute to a lack of representation of species found in the gut microbiome in AD infants. Several analyses in our study reported statistically insignificant results in contrast to published results; this may be due to insufficient power to reach statistical significance. Furthermore, small sample size may cause deviation from Chi-square test assumptions due to its sensitivity to sample size, producing inaccurate approximations (45).

Furthermore, there are inherent limitations of 16S rRNA amplicon sequencing, such as inability to revolve closely related species due to homology between sequences (46). Consequently, our analysis was primarily focused on the genus level, rather than the species level. Sequencing of hypervariable regions on the 16S rRNA may result in missed variations outside the selected region (7). In comparison to whole genome sequencing, major limitations of this approach are the decreased detection of diversity and accuracy of species identification (47).

Finally, reproducibility of our results is impacted by ambiguities introduced into the QIIME2 pipeline at decision-making points, including selection of a truncation length during denoising and sampling depth for rarefaction. A truncation length of 129 and a sampling depth of 26,745 was implemented in our analysis to maintain a Phred score of 38 and to maximize both feature and sample retention. Selection of these parameters was subjective; future user decisions may impact analysis results.

Conclusions In addressing the relationship between infant feeding method and AD onset, this study found feeding method and AD status to be significantly correlated. This finding supports what is currently known in the literature as both are heavily connected with the gut microbiota. We found that breastfed infants had lower alpha diversity than formula-fed infants. Furthermore, breastfeeding was found to favour the abundance of specific genera only in healthy infants. In comparison, formula feeding favours the abundance of specific genera in both AD and healthy infants. The present study validates previous literature that infant feeding method affects AD development, and provides evidence that this relationship may be linked through the gut microbiota.

Future Directions Our findings provide a basis for future research on the mechanistic relationship between infant feeding method and AD onset. A better understanding of the role that the gut microbiota plays in this relationship would be a critical step towards elucidating an explanatory cause for AD development. This includes a functional understanding of the

pathways that these differentially abundant taxonomic groups in healthy and AD infants contribute to, from which clearer links between these functions and their impact on the host, such as host immune development, can be drawn. As these taxonomic groups are part of larger communities, assessments of correlation networks can help reveal how these networks differ between healthy and AD infants, and whether feeding type can contribute to the changes between these networks. Additionally, it is of interest to further investigate how these differentially abundant taxa, identified in this study, participate within these networks and whether they contribute to clusters that perform different functions between healthy and AD infants. The gut microbiota is a promising target for therapeutic development and identification of biomarkers for disease diagnosis. Ultimately, future research may inform the production of fortified infant formulas, and families stand to benefit from improved feeding recommendations.

ACKNOWLEDGEMENTS

The authors would like to extend a warm thank you to Dr. Evelyn Sun, Dr. Steven Hallam, Dr. Stephan Koenig, and Zakhar Krekhno for their ongoing guidance, suggestions, and technical support throughout this project. Additionally, the authors would thank the University of British Columbia (UBC), Department of Microbiology and Immunology for providing the computational resources and funding for this project. Finally, the authors would like to thank Dr. Kyung Rhee and her research group at the University of California San Diego for their dataset PRJEB39437, which was used in this project. We would also like to thank two anonymous reviewers for constructive feedback on this manuscript.

REFERENCES

1. Williams HC. 2005. Clinical practice. Atopic dermatitis. N Engl J Med 352:2314–2324.
2. Silverberg JI, Gelfand JM, Margolis DJ, Boguniewicz M, Fonacier L, Grayson MH, Chiesa Fuxench ZC, Simpson EL, Ong PY. 2019. Pain is a common and burdensome symptom of atopic dermatitis in United States adults. J Allergy Clin Immunol Pract 7:2699-2706.e7.
3. Ong PY, Leung DY. 2006. Immune dysregulation in atopic dermatitis. Curr Allergy Asthma Rep 6(5):384-9.
4. Avena-Woods C. 2017. Overview of atopic dermatitis. Am J Manag Care 23:S115–S123.
5. David Boothe W, Tarbox JA, Tarbox MB. 2017. Atopic dermatitis: Pathophysiology. Adv Exp Med Biol 1027:21–37.
6. Thorsteinsdottir S, Stokholm J, Thyssen JP, Nørgaard S, Thorsen J, Chawes BL, Bønnelykke K, Waage J, Bisgaard H. 2019. Genetic, clinical, and environmental factors associated with persistent atopic dermatitis in childhood. JAMA Dermatol 155:50–57.
7. Zhang J, Chiodini R, Badr A, Zhang G. 2011. The impact of next-generation sequencing on genomics. J Genet Genomics 38:95–109.
8. van den Elsen LWJ, Garssen J, Burcelin R, Verhasselt V. 2019. Shaping the gut microbiota by breastfeeding: The gateway to allergy prevention? Front Pediatr 7:47.
9. Ma J, Li Z, Zhang W, Zhang C, Zhang Y, Mei H, Zhuo N, Wang H, Wang L, Wu D. 2020. Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: A study of 91 term infants. Sci Rep 10:15792.
10. Lee SY, Lee E, Park YM, Hong SJ. 2018. Microbiome in the gut-skin axis in atopic dermatitis. Allergy Asthma Immunol Res 10:354–362.
11. Zheng D, Liwinski T, Elinav E. 2020. Interaction between microbiota and immunity in health and disease. Cell Res 30:492–506.
12. Cioffi CC, Tavalire HF, Neiderhiser JM, Bohannan B, Leve LD. 2020. History of breastfeeding but not mode of delivery shapes the gut microbiome in childhood. PLOS ONE 15:e0235223.
13. Gdalevich M, Mimouni D, David M, Mimouni M. 2001. Breast-feeding and the onset of atopic dermatitis in childhood: A systematic review and meta-analysis of prospective studies. J Am Acad Dermatol 45:520–527.
14. Snijders BEP, Thijs C, Dagnelie PC, Stelma FF, Mommers M, Kummeling I, Penders J, van Ree R, van den Brandt PA. 2007. Breast-feeding duration and infant atopic manifestations, by maternal allergic status, in the first 2 years of life (KOALA study). J Pediatr 151:347-351.e2.
15. Laubereau B, Brockow I, Zirngibl A, Koletzko S, Gruebl A, Berg A von, Filipiak-Pittroff B, Berdel D, Bauer CP, Reinhardt D, Heinrich J, Wichmann H-E, Group for the GS. 2004. Effect of breast-feeding on the development of atopic dermatitis during the first 3 years of life—results from the GINI-birth cohort study. J Pediatr 144:602–607.

16. Schoetzau A, Filipiak-Pittroff B, Franke K, Koletzko S, Von Berg A, Gruebl A, Bauer CP, Berdel D, Reinhardt D, Wichmann H-E, German Infant Nutritional Intervention Study Group. 2002. Effect of exclusive breast-feeding and early solid food avoidance on the incidence of atopic dermatitis in high-risk infants at 1 year of age. Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol 13:234–242.
17. Wopereis H, Sim K, Shaw A, Warner JO, Knol J, Kroll JS. 2018. Intestinal microbiota in infants at high risk for allergy: Effects of prebiotics and role in eczema development. J Allergy Clin Immunol 141:1334-1342.e5.
18. Lee MJ, Kang MJ, Lee SY, Lee E, Kim K, Won S, Suh DI, Kim KW, Sheen YH, Ahn K, Kim BS, Hong SJ. 2018. Perturbations of gut microbiome genes in infants with atopic dermatitis according to feeding type. J Allergy Clin Immunol 141:1310–1319.
19. Reddel S, Del Chierico F, Quagliariello A, Giancristoforo S, Vernocchi P, Russo A, Fiocchi A, Rossi P, Putignani L, El Hachem M. 2019. Gut microbiota profile in children affected by atopic dermatitis and evaluation of intestinal persistence of a probiotic mixture. Sci Rep 9:4996.
20. Marrs T, Jo J-H, Perkin MR, Rivett DW, Witney AA, Bruce KD, Logan K, Craven J, Radulovic S, Versteeg SA, van Ree R, McLean WHI, Strachan DP, Lack G, Kong HH, Flohr C. 2021. Gut microbiota development during infancy: Impact of introducing allergenic foods. J Allergy Clin Immunol 147:613-621.e9.
21. Lau S, Gerhold K, Zimmermann K, Ockeloen CW, Rossberg S, Wagner P, Sulser C, Bunikowski R, Witt I, Wauer J, Beschorner J, Menke G, Hamelmann E, Wahn U. 2012. Oral application of bacterial lysate in infancy decreases the risk of atopic dermatitis in children with 1 atopic parent in a randomized, placebo-controlled trial. J Allergy Clin Immunol 129:1040–1047.
22. Kang Y, Cai Y, Pan W. 2018. Change in gut microbiota for eczema: Implications for novel therapeutic strategies. Allergol Immunopathol (Madr) 46:281–290.
23. Isolauri E, Kalliomäki M, Laitinen K, Salminen S. 2008. Modulation of the maturing gut barrier and microbiota: a novel target in allergic disease. Curr Pharm Des 14:1368–1375.
24. Feltis BA, Wiesner SM, Kim AS, Erlandsen SL, Lyerly DL, Wilkins TD, Wells CL. 2000. Clostridium difficile toxins A and B can alter epithelial permeability and promote bacterial paracellular migration through HT-29 enterocytes. Shock Augusta Ga 14:629–634.
25. Pearson K. 1900. X. On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. Lond Edinb Dublin Philos Mag J Sci 50:157–175.
26. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High resolution sample inference from Illumina amplicon data. Nat Methods 13:581–583.
27. Robeson MS, O'Rourke DR, Kaehler BD, Ziemski M, Dillon MR, Foster JT, Bokulich NA. 2020. RESCRIPt: Reproducible sequence taxonomy reference database management for the masses. preprint, Bioinformatics.
28. Faith DP. 1992. Conservation evaluation and phylogenetic diversity. Biol Conserv 61:1–10.
29. Pielou EC. 1966. The measurement of diversity in different types of biological collections. J Theor Biol 13:131–144.
30. Schroeder PJ, Jenkins DG. 2018. How robust are popular beta diversity indices to sampling error? Ecosphere 9:e02100.
31. Lozupone CA, Hamady M, Kelley ST, Knight R. 2007. Quantitative and qualitative β diversity measures lead to different insights into factors that structure microbial communities. Appl Environ Microbiol 73:1576–1585.
32. Lozupone C, Knight R. 2005. UniFrac: A new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71:8228–8235.
33. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res 41:D590.
34. Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H. 2019. Welcome to the Tidyverse. J Open Source Softw 4:1686.
35. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P, O'Hara B, Simpson G, Solymos P, Stevens H, Wagner H. 2015. Vegan: Community Ecology Package. R Package Version 22-1 2:1–2.
36. McMurdie PJ, Holmes S. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLOS ONE 8:e61217.
37. Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, Lozupone C, Zaneveld JR, Vázquez-Baeza Y, Birmingham A, Hyde ER, Knight R. 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5:27.
38. Lien TY, Goldman RD. 2011. Breastfeeding and maternal diet in atopic dermatitis. Can Fam Physician 57:1403–1405.
39. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW. 2000. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 30:61–67.
40. Zimmermann P, Curtis N. 2018. Factors influencing the intestinal microbiome during the first year of life. Pediatr Infect Dis J 37:e315–e335.
41. Nagayama M, Yano T, Atarashi K, Tanoue T, Sekiya M, Kobayashi Y, Sakamoto H, Miura K, Sunada K, Kawaguchi T, Morita S, Sugita K, Narushima S, Barnich N, Isayama J, Kiridooshi Y, Shiota A, Suda W,

Hattori M, Yamamoto H, Honda K. 2020. TH1 cell-inducing Escherichia coli strain identified from the small intestinal mucosa of patients with Crohn's disease. Gut Microbes 1–14.
42. Tang L, Zhou F. 2020. Inflammasomes in common immune-related skin diseases. Front Immunol 11: 882
43. Ling Z, Li Z, Liu X, Cheng Y, Luo Y, Tong X, Yuan L, Wang Y, Sun J, Li L, Xiang C. 2014. Altered fecal microbiota composition associated with food allergy in infants. Appl Environ Microbiol 80:2546–2554.
44. Fyhrquist N, Ruokolainen L, Suomalainen A, Lehtimäki S, Veckman V, Vendelin J, Karisola P, Lehto M, Savinko T, Jarva H, Kosunen TU, Corander J, Auvinen P, Paulin L, von Hertzen L, Laatikainen T, Mäkelä M, Haahtela T, Greco D, Hanski I, Alenius H. 2014. Acinetobacter species in the skin microbiota protect against allergic sensitization and inflammation. J Allergy Clin Immunol 134:1301-1309.e11.
45. Bergh D. 2015. Sample size and chi-squared test of fit—A comparison between a random sample approach and a chi-square value adjustment method using Swedish adolescent data, p. 197–211. In Zhang, Q, Yang, H (eds.), Pacific Rim Objective Measurement Symposium (PROMS) 2014 Conference Proceedings. Springer, Berlin, Heidelberg.
46. Bailén M, Bressa C, Larrosa M, González-Soltero R. 2020. Bioinformatic strategies to address limitations of 16rRNA short-read amplicons from different sequencing platforms. J Microbiol Methods 169:105811.
47. Ranjan R, Rani A, Metwally A, McGee HS, Perkins DL. 2016. Analysis of the microbiome: Advantages of whole genome shotgun versus 16S amplicon sequencing. Biochem Biophys Res Commun 469:967–977.