Gut microbiome characteristics in mothers and infants according to the presence of atopic dermatitis

Myong Soon Sung[1], Yujin Choi[2], Hyunjoon Park[3], and Chul Sung Huh[3]

[1]Soonchunyang University Gumi Hospital
[2]CHA Gumi Medical Center, CHA University School of Medicine
[3]Research Institute of Eco-friendly Livestock Science, Institute of Green-Bio Science and Technology, Seoul National University

March 8, 2021

Abstract

Background: The role of the gut microbiome in the onset and development of atopic dermatitis (AD) has been postulated. Therefore, we investigated the gut microbial compositions in infants with and without AD, and compared it to the gut bacterial flora of their mothers. Methods: This was a prospective and cross-sectional study. Among 44 pairs of mothers and children, we selected infants who were born via full-term normal vaginal delivery and that had no history of antibiotic or probiotic use, and infection during the first three months of life. The 15 pairs, consisting of nine healthy infants and six AD infants, were included in this study. Fecal samples of mothers and infants were analyzed within 30 days of delivery and at 12 months of age. Microbes in the fecal samples of mothers and infants were subjected to analysis of 16S rRNA amplicon sequencing. Results: Abundance of specific taxonomic groups was notably different, but microbial diversity and phylogenetic distances were not significantly different in either maternal or infant groups according to the presence of infant AD. A total of 12 species were selected as differential species in infants with AD compared to healthy infants. Six species were significantly different in the mothers of infants with AD compared to the mothers of healthy infants. Akkermansia muciniphila was only detected in healthy infants and their mothers. Conclusions: These data indicated that the presence of Akkermansia muciniphila in mothers and children after vaginal delivery is associated with the onset and development of AD.

Gut microbiome characteristics in mothers and infants

according to the presence of atopic dermatitis

Myongsoon Sung[1)], Yujin Choi[2)], Hyunjoon Park[3)], Chul Sung Huh[3, 4)]

[1)] Department of Pediatrics, Soonchunhyang University Gumi Hospital, Gumi, Republic of Korea

[2)] Department of Pediatrics, CHA Gumi Medical Center, CHA University School of Medicine, Gumi, Republic of Korea

[3)] Research Institute of Eco-friendly Livestock Science, Institute of Green-Bio Science and Technology, Seoul National University, Pyeongchang, Republic of Korea

[4)] Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang, Republic of Korea

Running title

Gut microbiome in mothers and infants

Corresponding author

Professor Chul Sung Huh, Ph D

Research Institute of Eco-friendly Livestock Science, Institute of Green-Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 25354, Republic of Korea

Phone: +82-33-339-5723; Fax: +82-33-339-5689

Email: cshuh@snu.ac.kr

Abstract

Background: The role of the gut microbiome in the onset and development of atopic dermatitis (AD) has been postulated. Therefore, we investigated the gut microbial compositions in infants with and without AD, and compared it to the gut bacterial flora of their mothers.

Methods: This was a prospective and cross-sectional study. Among 44 pairs of mothers and children, we selected infants who were born via full-term normal vaginal delivery and that had no history of antibiotic or probiotic use, and infection during the first three months of life. The 15 pairs, consisting of nine healthy infants and six AD infants, were included in this study. Fecal samples of mothers and infants were analyzed within 30 days of delivery and at 12 months of age. Microbes in the fecal samples of mothers and infants were subjected to analysis of 16S rRNA amplicon sequencing.

Results: Abundance of specific taxonomic groups was notably different, but microbial diversity and phylogenetic distances were not significantly different in either maternal or infant groups according to the presence of infant AD. A total of 12 species were selected as differential species in infants with AD compared to healthy infants. Six species were significantly different in the mothers of infants with AD compared to the mothers of healthy infants. *Akkermansia muciniphila* was only detected in healthy infants and their mothers.

Conclusions: These data indicated that the presence of*Akkermansia muciniphila* in mothers and children after vaginal delivery is associated with the onset and development of AD.

Keywords : Gut microbiome; atopic dermatitis; infant; mother;

Akkermansia muciniphila

Key Messages

* Study examined 15 pairs of mothers and infants and fecal samples of mothers and infants were analyzed within 30 days of delivery and at 12 months of age.

* All infants had a full-term vaginal delivery and were examined for controllable factors such as breastfeeding/mixed feeding, probiotic consumption, and antibiotic treatment.

* *Akkermansia muciniphila* was only detected in healthy infants and their mothers.

Abbreviations

CI, confidence interval; OR, odds ratio; aOR, adjusted odds ratio; AD, atopic dermatitis;

SCORAD, SCORing Atopic Dermatitis; SPT, skin prick testing; IAD, infant AD; IHC, infant

healthy controls ; *D.p* , *Dermatophagoides pteronyssinus* ;*D.f* , *Dermatophagoides farina;* IRBs, Institutional Review Boards; *A. muciniphila, Akkermansia muciniphila* ;

1 INTRODUCTION

Atopic dermatitis (AD) is one of the most common types of dermatosis in infants.[1] AD in children affects 17-24% of the total pediatric population, and developing during the first six months of life in 45% of the affected children and by five years of age in 85% of those affected.[1] However, the underlying pathology of

AD is heterogeneous and causes are known to be a poorly defined mix of the innate and adaptive immune responses.[2]

The gut encompasses diverse and dynamic microbial ecology, which is linked to human health and various diseases.[3] Since the role of the gut microbiome in the early development of the immune system has been reported, its impact on allergic diseases, including AD, has been explored.[4] The first 12 months of life is a critical period of microbial colonization in the gastrointestinal tract.[5] Recent studies have shown the differences in the gut microbiome of infants with AD and those without AD.[5-7] Moreover, temporal relationships of the gut microbiota with multi-sensitized atopy and IgE-associated eczema have been reported.[7] Various factors affect the microbial colonization of the infants' gut, such as the delivery mode, feeding method, weaning period, regular diet, probiotics consumption, and antibiotic treatments.[8, 9] Furthermore, these factors may influence the development of AD in infants.[10-12]Additionally, perturbations of the mother' s gut microbiome may be associated with the poor development of the infant's immune system and with a higher risk of AD.

In this study, we hypothesized that the specific status of the mother's gut microbiome might be associated with the development of the immune system and the risk of AD in infants. Additionally, the natural course of AD in infants is characterized by development during an early stage, from the first 6 months to 12 months, so we aimed to investigate the mother and infant- associated early microbiome markers to predict AD in infants.

2 METHODS

2.1 Study population

This was a prospective and cross-sectional study that enrolled maternal and child pairs from December 2016 to December 2018. The study involved the SCORing Atopic Dermatitis (SCORAD) score assessment at six and 12 months of age and skin prick testing (SPT) and fecal sampling at 12 months of age for the infant. Also, fecal samples from the mother were collected within 30 days of delivery. This study protocol was approved by the Institutional Review Boards (IRBs) of the CHA Medical Center (IRB No. 2016-1137). Written informed consent was obtained from the parents or guardians of all participants following a detailed explanation of the study.

A total of 44 pairs of mothers and children provided their consent to participate in the study. We selected infants who were born via full-term normal vaginal delivery and that had no history of antibiotic or probiotic use, and infection during the first three months of life. They were fed with a combination of breast milk and formula or breast milk for the first six months of life and started solid food intake within six months of age. Additionally, the mothers did not consume any probiotics during pregnancy, and all families lived with no pets. Infants and mothers had no history of antibiotic use before fecal sampling for seven days. Eventually, this study population consisted of 15 maternal and child pairs from nine healthy infants and six AD infants.

In the present study, the infant AD (IAD) group was identified through a comprehensive evaluation of clinical history, including lifetime symptoms of AD via questionnaire and physical examination by a pedestrian specializing in allergies (Dr. Sung) at six and 12 months of age. The infant healthy controls (IHC) group consisted of children who had a SCORAD score of 0 on physical examination by the same pediatrician and no history of allergic diseases (AD, allergic rhinitis, or asthma) as assessed in the questionnaire during the same period.

2.2 Questionnaire and SCORAD score

The diagnosis of allergic disease (AD, allergic rhinitis, and asthma) was made based on the Korean ISAAC questionnaire, a standardized method of evaluating allergic diseases in epidemiologic studies in Korea.[13] In accordance with the criteria of Hanifin and Rajka,[14] a pediatrician (Dr. Sung) made the diagnosis of AD after physical examination of each child, and calculated the SCORAD scores at six and 12 months of age. The AD group was divided into three classes based on the severity of AD: mild (< 25), moderate (25 - 50), and severe (> 50).[15]

2.3 Measurement of atopy

SPT was performed with standardized allergen extracts and control solutions from LaForma (Milan, Italy) on the volar surface of both arms. Subjects were tested for sensitivity to the common aeroallergens: house dust mites (HDM; *Dermatophagoides pteronyssinus (D.p),Dermatophagoides farina (D.f)*), and four common food allergens (eggs, milk, peanut, and soy). SPT was performed on infants with AD between12 and 13 months of age, and a positive SPT was defined as one with a mean wheal diameter that was 3 mm or greater compared to the positive control. Atopy was defined as the presence of positive SPT.

2.4 Fecal sample preparation and genomic DNA extraction

Fecal samples were collected from mother within30 days of childbirth and, from their infants at 12 months of age. Thirty fecal samples were collected from nine pairs of non-AD healthy infants (IHC group) and their mothers (MHC group), and six pairs of AD infants (IAD group) and their mothers (MAD group). The samples were obtained in bottles containing DNA/RNA Shield (Zymo Research, Irvine, CA, USA) at an equal volume (w/v) and stored immediately at –80°C. Cellular DNA extraction was conducted using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research), according to the manufacturer's instructions.

2.5 Miseq 16S rRNA amplicon sequencing

Sequencing libraries of the V3–V4 regions of the 16S bacterial rRNA gene were constructed following Illumina's protocol.[16]Two-step PCR with two clean-up procedures was conducted and the obtained 16S rRNA amplicons were sequenced on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) by Chun-Lab Inc. (Seoul, South Korea). Detailed amplicon and index PCR conditions were as follows: amplicon PCR (95°C for 3 min; 25 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s; and 72°C for 5 min), index PCR (95°C 3 min; 8 cycles of 95°C 30 s, 55°C 30 s, and 72°C 30 s; and 72°C for 5 min).

2.6 Microbiome analysis

In the Quantitative Insight into Microbial Ecology 2 (QIIME 2) pipeline,[17] the paired-end sequenced reads were demultiplexed and quality controlled using the DADA2 algorithm to obtain an amplicon sequence variant (ASV) table. Phylogenetic trees were created, and taxonomy was annotated with the naive Bayes classifier against the Greengenes database. The obtained files were imported into R software (www.r-project.org) and transformed to phyloseq objects for the subsequent analysis.[18] Cho1 and Shannon indices were applied to measure microbial richness and diversity. To calculate the phylogenetic distances between groups, weighted-UniFrac and generalized UniFrac methods were used, and the sample distribution was determined using the MDS/PCoA and NMDS methods.[19] To determine whether group separations were significant, ADONIS and permutational MANOVA (PERMANOVA) tests were conducted. The differential abundances of specific microbial taxa were analyzed using the DESeq2 package.[20]

2.7 Statistical analysis

SPSS Statistics ver. 19.0 (IBM Co., Armonk, NY, USA) was used for all statistical analyses. Values are reported as the mean ± standard deviation. Scale variables were analyzed using Fisher's exact test, and continuous variables were analyzed using the Mann Whitney U-test.

A correlation map was constructed using the MeV software package (*http://www.tm4.org/*) based on correlation coefficient values calculated using PASW Statistics 18 software. Statistical significance was defined as $P < 0.05$. Relative risk (RR) ratios and corresponding 95% CIs were calculated using log-binominal regression with the maximum likelihood estimation in R software. The different bacterial taxa and KEGG ortholog categories between the groups were determined using the Kruskal-Wallis rank sum test in R (*www.r-project.org*).

3 RESULTS

3.1 Characteristics of the study subjects

A total of 15 infants and mothers participated in the study: nine in the HC group and six in the AD group (Table 1). All infants had a full-term normal vaginal delivery and no history of antibiotic or probiotic use and infection during the first three months of life. They started solid-food intake within six months old. They fed with a combination of breastmilk and formula or breast milk for the first six months of life, and there was no significant difference between the HC and AD groups with regard to breastfeeding duration or mixed feeding. The overall severity of AD was found to be mild and moderate-severe in five infants (83.3%) and one infant (16.7%) in the AD group, respectively. Among the six infants with AD, three infants had sensitization to allergens, and the most common allergen was eggs.

The mother subjects had no probiotic consumption during pregnancy, and all families lived without pets (Table 1). There was no statistically significant difference between the HC and AD groups concerning allergic diseases and diet in pregnancy. In the AD group, two mothers had a history of antibiotic use during pregnancy, but there was no statistically significant difference between the two groups ($P = 0.143$).

3.2 Maternal gut bacterial differences based on child AD diagnosis

At the phylum level, there were changes in gut microbial composition of maternal individuals (FIGURE 1A). Both mother groups showed high populations of *Firmicutes* in the gut (FIGURE 1B). The relative abundances (RAs) of *Bacteroidetes* and *Actinobacteria* were similar between the maternal groups (FIGURE 1C and 1D). The population of *Proteobacteria* was significantly higher in the MAD group (0.37%) than in the MHC group (0.09%) ($P = 0.0004$) (FIGURE 1E). Of note, the *Verrucomicrobia* phylum was detected only in the MHC group (RAs = 1.38% and $P < 0.0001$) (FIGURE 1F). There were no significant differences in bacterial composition with regard to Chao1 and Shannon diversity between maternal groups (Supplementary FIGURE S1A). In beta diversity analysis, sample distributions in the maternal groups showed partial differences without significance (F value = 0.852, R squared = 0.051) (Supplementary FIGURE S1B and S1C).

3.3 gut bacterial differences based on AD diagnosis

The characteristics of the infant gut microbial composition are shown in FIGURE 2. A total of 15 infants were included, comprising nine healthy controls and six AD cases, and they showed individual patterns of the gut microbiome (FIGURE 2A). Among the assigned phyla, *Firmicutes* ,*Bacteroidetes* , and *Actinobacteria* were predominant in all infant faeces (FIGURE 2B-D). The IHC group showed relatively higher abundances of *Actinobacteria* , but the difference was not statistically significant ($P = 0.4614$) (FIGURE 2D). There was no considerable difference in the RAs of *Proteobacteria* between the infant groups (FIGURE 4E). In particular, the population of*Verrucomicrobia* was detected only in the IHC group (2.312%) ($P < 0.0001$) (FIGURE 2F). The infant groups showed no significant differences in the alpha (Supplementary FIGURE S2A) and beta diversity (Supplementary FIGURE S2B and S2C), and considerable phylogenetic separation was not observed ($F = 0.592$, R squared = 0.057).

3.4 Differential abundance in specific microbial taxonomic group

In our results, there were no notable differences between groups in microbial richness/diversity and the RAs at the phylum level (FIGURE 1 and 2), except for *Verrucomicrobia* (FIGURE 1F and 2F) and*Proteobacteria* (FIGURE 1E). Therefore, we sorted the top 100 most abundant bacterial taxa (FIGURE 3A) and applied the DESeq2 method to find differential microbial taxa between groups using statistical criteria (BaseMean > 1 and $P < 0.05$) (Table 2).

In the infant group comparison, seven operational taxonomic units (OTUs) (classified as *Bifidobacterium* , *B. breve* ,*Clostridium paraputrificum* , uncultured*Clostridiales* , uncultured *Lachnospiraceae* , and*Akkermansia muciniphila*) were significantly lower in the IAD group than in the IHC group, while five OTUs (*Bacteroides* ,*Dorea longicatena* , *Faecalibacterium* , and*Ruminococcus lactaris*) were significantly higher in the IAD group. In the case of the maternal groups, *Coprococcuseutactus* , *Ruminococcus lactaris* , uncultured*Clostridiales* , and *Akkermansia muciniphila* were significantly lower in the MAD group than in the MHC group, but only*Prevotella* was a differentially higher taxon in the MAD group. Among the selected

taxa, *Akkermansia* , which belongs to the phylum *Verrucomicrobia* , showed similar patterns in the maternal and infant groups based on the presence of AD in children (FIGURE 3B and 3C).

4 DISCUSSION

In this study, we compared the composition and genes of the gut microbiome in healthy infants and those with AD at 12 months of age, as well as in their mothers within 30 days of birth. Interestingly, we demonstrated that *Akkermansia muciniphila* (*A. muciniphila*) was present in the gut microbiome of healthy children in early life and their mothers, but not in children with AD and their mothers, which supports the previous findings of the gut microbiome's role in the onset of AD.

It has been established that the maturation of the gut microbiome from prepartum to three years of age is influenced by various maternal determinants, such as host genetics, feeding method, maternal diet, maternal infections, and delivery mode.[21, 22] From these data, we postulated that the difference in the gut microbiome according to the infant's AD might be derived from the mother's gut flora. Thus, we analyzed whether the pattern of gut microbial composition in the mother groups was similar to the composition detected in the infant groups.

A. muciniphila , which was first isolated in 2004,[23] is a probiotic species associated with human health and diseases, such as obesity, type 2 diabetes, and colorectal cancer.[24] Some recent studies demonstrated that low or absent of *A. muciniphila* in the gut microbiome of children with AD,[25] which might play a role in IgE-mediated atopic disease. Moreover, *A. muciniphila* contributes to the regulation of mucosal innate immune response due to its mucus-degrading characteristics.[25] According to a recent study, the proportion of *A. muciniphila* was higher in children with transient AD than in children with non-AD or persistent AD.[26] However, in our study, the proportion of *Akkermansia* was higher in children with AD than in children without AD because we included only the persistent AD subtype. Therefore more researches is needed to clarify these relationships.[26]

Meanwhile, the abundance of some taxa, including *Bifidobacterium, Clostridium* , *Lachnospiraceae,* and *Faecalibacterium* , has been commonly reported in children with AD, [21, 27] which is in agreement with the present study, even though there has been an ongoing debate about gut microbiomes associated with allergic disease. It is well known that *Bifidobacterium* , one of the major genera in an infant's gut, is less abundant in infants with AD, in accordance with the present findings. However, some researchers suggest that oligosaccharide type and composition affect the composition of *Bifidobacterium spp* . in the gut according to the feeding method and thus lead to the development of atopic disorders.[21, 27, 28] However, this data is different from our data, in which all infants had breast milk feeding or mixed feeding, but *Bifidobacterium* was significantly lower in the children with AD. According to a longitudinal study of the microbiome of infants with up to 200 days of age, the populations of *Faecalibacterium* and *Lachnospiraceae* in infants with AD changed inversely.[29]

Some studied suggested that maternal diet and infection during pregnancy influence the infant's gut microbiome.[21, 22, 29] The perturbation process results in changes in the host-microbiome biodiversity and metabolic activities. It has been associated with greater susceptibility to immune-mediated disorders, such as AD, later in life.[22,29] In this study, in the MAD group, two mothers had antibiotic medication during pregnancy, but there was no significant difference between the MAD and MHC groups, probably due to the small sample size. The sample size used in this analysis was relatively small because of the exclusion criteria applied (normal delivery with full term, breast milk feeding, and antibiotic medication); therefore, we need to be careful with the generalization of these results. The AD severity was mostly mild, as our subjects were sourced from a general population-based prospective cohort study not patient with AD. Also, the human intestinal microbiota comprised bacteria and fungi, but we did not consider the roles of fungi. Further studies are necessary to resolve these limitations, including a replication study using most of our current subjects and functional studies to assess these phenomena mechanistically.

However, this study has several strengths. First, our study subjects were recruited from a prospective cohort study. Second, we analyzed stool samples from children who had not received antibiotics for the first three months and mothers and infants had no history of antibiotic usage before fecal sampling. The history of

antibiotic usage could have affected the composition of the gut microbiome and the relationship between mother-child gut microbiomes. Third, the AD phenotype, in terms of the natural course of this disorder, was assessed by the same pediatric allergist twice. We analyzed the follow-up data regarding the progression of AD, which was recorded by a pediatric allergist from birth to 12 months of age, and performed 16s rRNA sequencing.

We found an abundance of *Akkermansia* only in healthy maternal-child pairs among the taxa analyzed, but not in infants with AD and their mothers. Based on previous studies, we assume that the less abundant *Akkermansia* in infants with AD may be derived from their mother's gut flora, which may have affected the onset or development of AD in infants. In conclusion, the gut microbiome and its influence on innate immune development in infants and mothers play a crucial role in infants with AD. Further studies are needed to identify the association and roles of *Akkermansia* in the infant gut and development of atopic disorders.

ACKNOWLEDGMENTS

Grant funding was provided by the Korean Academy Asthma, Allergy and Clinical Immunology Research Fund and the Soonchunhyang University Research Fund.

CONFLICT OF INTEREST

The authors have indicated they have no potential conflicts of interest to disclose.

REFERENCES

1. Laughter D, Istvan JA, Tofte SJ, et al. The prevalence of atopic dermatitis in Oregon schoolchildren. *J Am Acad Dermatol* . 2000; **43** : 649-655.

2. Shreiner AB, Kao JY, Young VB. The gut microbiome in health and in disease. *Curr Opin Gastroenterol.* 2015; **31** : 69-75.

3. Backhed F, Roswall J, Peng Y, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell Host Microbe.*2015; **17** :690-703.

4. Wopereis H, Oozeer R, Knipping K, et al. The first thousand days- intestinal microbiology of early life: establishing a symbiosis. *Pediatr Allergy Immunol* . 2014; **25** :428-438.

5. Fujimura KE, Sitarik AR, Havstad S, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med* . 2016; **22** :1187-1191.

6. Zheng H, Liang H, Wang Y, et al. Altered gut microbiota composition associated with eczema in infants. *PLoS One.* 2016; **11** : e0166026.

7. Arrieta MC, Stiemsma LT, Dimitriu PA,et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci Transl Med* .2015;**7** : 307ra152.

8. Vinod K Gupta, Sandip Paul, Chitra Dutta. Geography, Ethnicity or Subsistence-Specific Variations in Human Microbiome Composition and Diversity. *Front Microbiol.* 2017; **8** :1162.

9. Zhernakova, A., Kurilshikov, A., Bonder, M. J, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity.*Science.* 2016; **352** : 565–569.

10. Milani C, Duranti S, Bottacini F et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. *Microbiol Mol Biol Rev* . 2017;**81** : e00036-17.

11. Eun Lee, Byoung-Ju Kim, Mi-Jin Kang et al. Dynamics of Gut Microbiota According to the Delivery Mode in Healthy Korean Infants. *Allergy Asthma Immunol Res.* 2016 ;**8**: 471- 477.

12. Rutayisire E, Huang K, Liu Y, et al. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterol* . 2016;**16** :86.

13. Lee SI, Shin MH, Lee HB, et al. Prevalences of symptoms of asthma and other allergic diseases in Korean children: a nationwide questionnaire survey. *J Korean Med Sci.* 2001:**16** :155-164.

14. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol*
Suppl (Stockh) . 1980:**92** : 44-47.

15. Oranje AP, Glazenburg EJ, Wolkerstorfer A, et al. Practical issues on interpretation of scoring atopic dermatitis: The SCORAD index, objective SCORAD and the three-item severity score. *Br J Dermatol* . 2007;**57** :645-648.

16. Amplicon PCR, Clean-Up PCR, Index PCR. 16S metagenomic sequencing library preparation. Available online: https://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry documentation/16s/16s

17. Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol.* 2019; **37**:852-857.

18. McMurdie, P.J, Holmes, S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* . 2013;**8** : e61217

19. Chen, J, Bittinger, K, Charlson, ES, et al. Associating microbiome composition with Environmental covariates using generalized UniFrac distances.*Bioinformatics* . 2012; **28** ; 2106-2113.

20. Love, MI, Huber, W, Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* . 2014; **15** : 550.

21. Wopereis H, Sim K, Shaw A, et al. Intestinal microbiota in infants at high risk for allergy: Effects of prebiotics and role in eczema development. *J Allergy Clin Immunol.* 2018;**141**:1334-1342.e5.

22. Peroni DG, Nuzzi G, Trambusti I, et al. Microbiome Composition and Its Impact on the Development of Allergic Diseases. *Front Immunol* . 2020;**11** :700.

23. Dubourg G, Cornu F, Edouard S, et al. First isolation of Akkermansia muciniphila in a blood-culture sample. *Clin Microbiol Infect* . 2017; **23**:682-683.

24. Zhang T, Li Q, Cheng L, et al. Akkermansia muciniphila is a promising probiotic. *Microb Biotechnol* . 2019; **12** :1109-1125.

25. Derrien M, Van Baarlen P, Hooiveld G, et al. Modulation of Mucosal Immune Response, Tolerance, and Proliferation in Mice Colonized by the Mucin-Degrader Akkermansia muciniphila. *Front Microbiol.* 2011; **2** : 166.

26. Park YM, Lee SY, Kang MJ et al. Imbalance of Gut Streptococcus, Clostridium, and Akkermansia Determines the Natural Course of Atopic Dermatitis in Infant. *Allergy Asthma Immunol Res.* 2020; **12** :322-337.

27. Lee MJ, Kang MJ, Lee SY, et al. Perturbations of gut microbiome genes in infants with atopic dermatitis according to feeding type. *J Allergy Clin Immunol* . 2018;**141** : 1310-1319.

28. Matsuki, T., Yahagi, K., Mori, H. et al. A key genetic factor for fucosyllactose Utilization affects infant gut microbiota development. *Nat Commun* . 2016; **7** : 11939.

29. Galazzo G, van Best N, Bervoets L, et al. Development of the Microbiota and Associations With Birth Mode, Diet, and Atopic Disorders in a Longitudinal Analysis of Stool Samples, Collected From Infancy Through Early Childhood. *Gastroenterology.* 2020 ;**158** : 1584-1596.

Hosted file

`2020.03.03 Table_\selectlanguage{english}su\selectlanguage{english}jeong.pdf` available at https://authorea.com/users/400143/articles/512503-gut-microbiome-characteristics-in-mothers-and-infants-according-to-the-presence-of-atopic-dermatitis

Hosted file

`Figure summary.21.03.03.pptx` available at https://authorea.com/users/400143/articles/512503-gut-microbiome-characteristics-in-mothers-and-infants-according-to-the-presence-of-atopic-dermatitis