Unravelling the Gut Microbiota of Cow's Milk–Allergic Infants, Their Mothers, and Their Grandmothers

Mera-Berriatua L[1,*], Zubeldia-Varela E[1,2,*], Martín-Antoniano IA[3,4], López de Maturana E[1,4], Rojo D[2], Bazire R[5], Cabrera-Freitag P[6], Barker-Tejeda TC[1,2], Ubeda C[7,8], Barber D[1], Francino MP[8,9], Ibáñez-Sandín MD[5,**], Pérez-Gordo M[1,**]

[1]Institute of Applied Molecular Medicine (IMMA), Department of Basic Medical Sciences, Facultad de Medicina, Universidad San Pablo-CEU, CEU Universities, ARADyAL, Madrid, Spain

[2]Centre for Metabolomics and Bioanalysis (CEMBIO), Department of Chemistry and Biochemistry, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, Boadilla del Monte, Madrid, Spain

[3]Institute of Applied Molecular Medicine (IMMA), Department of Clinical Medical Sciences, Facultad de Medicina, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain

[4]Genetic and Molecular Epidemiology Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

[5]Department of Allergy, H. Infantil Universitario Niño Jesús, FibHNJ, ARADyAL- RETICs Instituto de Salud Carlos III, IIS-P, Madrid, Spain

[6]Pediatric Allergy Unit, Allergy Service, Hospital General Universitario Gregorio Marañón, Gregorio Marañón Health Research Institute (IiSGM), Madrid, Spain

[7]Fundació per al Foment de la Investigació Sanitària i Biomèdica de la Comunitat Valenciana (FISABIO), Valencia, Spain

[8]CIBER en Epidemiología y Salud Pública, Madrid, Spain

[9]Joint Research Unit in Genomics and Health, Fundació per al Foment de la Investigació Sanitària i Biomèdica de la Comunitat Valenciana (FISABIO) and Institut de Biologia Integrativa de Sistemes (Universitat de València / Consejo Superior de Investigaciones Científicas), València, Spain

**These authors contributed equally.*

***These authors contributed equally.*

J Investig Allergol Clin Immunol 2022; Vol. 32(5): 395-398
doi: 10.18176/jiaci.0781

Key words: Gut microbiota. Food allergy. Cow's milk allergy. 16S rRNA gene sequencing. Intergenerational cohort.

Palabras clave: Microbiota intestinal. Alergia alimentaria. Alergia a leche de vaca. Secuenciación del gen ARNr 16S. Cohorte intergeneracional.

The gut microbiome constitutes a highly complex ecosystem in which bacteria are the most prominent components. Around 70% of primary colonization of the gut microbiota is maternal in origin [1], and the first 1000 days of life are crucial for the development of the intestinal microbiota [2]. Despite its early formation, the gut microbiota is highly dynamic and dependent on host-associated confounding factors such as age, diet, antibiotics, lifestyle, and environmental conditions [3,4]. Alterations in gut microbiota have been described in people with different types of allergy, including cow's milk allergy (CMA) [5-7].

CMA is one of the most prevalent types of food allergy in children, although 80% of patients achieve tolerance by the age of 4 years [8,9]. The etiology and pathophysiology of the disease remain unclear, as does the role of abnormalities in the infant's microbiota. One possible explanation could be that the mother's gut microbiota, once transmitted to offspring, may predispose infants to allergy. This study aimed to investigate the composition of the gut microbiota and its relationship with the onset of CMA in infants over 3 generations.

The study population consisted of 148 participants recruited in the Allergy Departments of Hospital Universitario Infantil Niño Jesús and Hospital General Universitario Gregorio Marañón and 5 health centers in Madrid, Spain (50 infants [16 healthy controls and 34 with CMA], their 50 mothers, and their 48 maternal grandmothers).

The study was approved by the Regional Ethics Committee for Clinical Research of Hospital Universitario Infantil Niño Jesús in Madrid (R-0004/17) according to the ethical guidelines outlined in the Declaration of Helsinki and its revisions. All participants provided their informed consent.

Infants were classified into 2 groups: allergic infants and control infants. Inclusion and exclusion criteria, fecal sample collection (Figure S1), analysis of epidemiological variables, and 16S rRNA gene sequencing are detailed in Supplementary Information.

The characteristics of the 50 infants, including variables for their mothers and grandmothers are shown in Table S1. Infant cases and controls were similar in terms of age (4.93 vs 5.00 months, respectively) and sex distribution. A highly significant association was found between allergy and type of feeding at sample collection in the infants (P<.01, Table S1). Allergic mothers were 5 times more likely to have an allergic child than a nonallergic child (P=.03) (Figure S2). Interestingly, Pali-Schöll et al [10] found that the degree of risk for allergy appears to be directly related to the family history of allergy and especially to maternal atopy. Furthermore, the smoking habits of the mothers (P=.019) and the grandmothers (P=.077) were associated with infant allergy according to both the univariate and the multivariate analysis (Table S2). Nonsmoking mothers were 90% less likely to have allergic infants than smokers. Likewise, nonsmoking grandmothers were associated with a decreased risk of having an allergic infant (OR=0.28; P=.05).

16S rRNA gene sequencing was performed on samples from infants aged between 4 and 6 months. A total of 19 523 010 sequences were generated; of these, 12 955 391 remained after filtering for quality and length and removal of chimera, resulting in 9641 amplicon sequence variants (ASVs) for the gut microbiome. Supplementary Figures S3A and S3B show the relative abundance of the bacterial phyla and families found in individuals of the 3 generations according to allergy. The composition of the fecal microbiome differed between adults and infants (PERMANOVA, P=.017). Analysis of α diversity (Shannon diversity index) and richness revealed a statistically significant increase in adults compared with infants (P<.001) (Figure S4).

Differences regarding abundant bacterial families and ASVs between the infant groups were identified using the ANCOM-II method. Interestingly, the relative abundance of Prevotellaceae and Acidaminococcaceae, 2 of the families with the lowest relative abundances, differed significantly between allergic and control infants (Supplementary Figure S3C). However, no significant differences were detected between allergic and nonallergic mothers or grandmothers. The most remarkable differences due to the feeding regimen of infants were observed between infants fed with hydrolysate and those fed with formula milk (PERMANOVA, P=.005), namely, an increased presence of Prevotellaceae in the formula group (Figure S3D). Previous studies have shown that a decrease in this genus in the lung microbiota is associated with asthma and chronic obstructive pulmonary disease [11]. In other studies, the increased abundance of *Prevotella* has been associated with rheumatoid arthritis [12] and inflammatory bowel disease [13]. The distinct gut microbiota composition associated with infants' diet is shown in Figure S5. As the formula milk ingested by infants was not the same in all cases, the type and brand were requested in the questionnaires given to the families. We observed no statistically significant association with any of the brands or the marked increase in certain bacteria. The significant association between the feeding regimen and allergy in infants may be explained by the fact that for most of the infants diagnosed with CMA, feeding was changed to hydrolysate either alone or combined with breast milk (~70%) as part of regular clinical practice. In contrast, most of the controls were fed with formula milk. Therefore, it is very difficult to separate both variables, as each infant's diet was conditioned by their allergic status.

The recruitment of a larger number of breastfed control infants would have been preferable to overcome this bias in the diet. Comparison of microbiota between formula-fed and breastfed control infants was not possible owing to the low number of breastfed control infants. However, it was difficult to recruit more breastfed controls because of the reluctance of nonallergic families to participate in the study. In addition, in Spain, formula milk is usually introduced when infants are 4 months old, immediately after maternity leave. Consequently, the number of breastfed controls that fulfilled the inclusion criteria was reduced. It is also worth mentioning that allergic infants with a mixed feeding regimen of breast milk and formula milk (Table S1) did not tolerate formula milk proteins. They ingested formula milk before the allergic reaction occurred, and samples were collected the following day.

Aiming to investigate the dissimilarity between infants, mothers, and grandmothers of the same family, we computed the Bray-Curtis distance between infants and mothers (I-M), mothers and grandmothers (M-G), and infants and grandmothers (I-G). Figure S3E shows that the M-G distance was shorter than the I-M and I-G distances, consistent with the close similarities between adult microbiotas and the differences with the infant gut microbiota. Additionally, the distance between members of the same family was similar, regardless of allergy, suggesting similar inheritance and development of the microbiota in healthy and allergic infants.

Several authors have reported the association between age and the human microbiome [14], although very few reported intergenerational results [15]. Ours is the first longitudinal study to present results for 3 generations with fecal

microbiome data. Its main strengths include the heterogeneity of the samples, rigorous determination of CMA, and the use of high-throughput, culture-independent techniques for identification of microbiota. We provide important insights into the transgenerational risk factors for infant allergy and into microbiome composition. We identified maternal smoking as a risk factor in the development of CMA allergy. We show that the Prevotellaceae family was significantly more frequent in control infants and formula-fed infants than in allergic infants and hydrolysate-fed infants, respectively. However, it was not possible to assign those differences to either the infants' allergy or their feeding regimen, since samples were collected at only 1 timepoint and once CMA had appeared. We did not find a bacterial signature transmitted by the maternal route that predisposed to CMA in infants at the timepoints assessed. Further prospective longitudinal studies with follow-up over close intervals will be necessary to investigate the presence of such a signature.

Acknowledgments

We would like to thank all the institutions and hospitals involved: Institute of Applied Molecular Medicine (IMMA, San Pablo CEU University, Madrid), Sequencing and Bioinformatics Service (FISABIO, Valencia), Hospital Universitario Infantil Niño Jesús (Madrid, Spain), Hospital Universitario Gregorio Marañón, Centro de Salud (CS) Eloy Gonzalo (Feliciano López, Ana María López Madrazo, Sonia Luna Ramírez), CS Baviera (Mercedes Velez García-Nieto), CS Ibiza (Alberto Morlan Sala), CS Justicia (Mª Rosario Antón Jiménez), CS Valleaguado (Paloma Ortiz Ramos), and CS Goya (Yolanda Martín Peinador). All the authors are grateful for the help received from all the IMMA researchers who participated in sample processing.

Funding

This work was supported by Instituto de Salud Carlos III (PI17/01087) and Fundación Sociedad Española de Alergia e Inmunología Clínica (FSEAIC_2016). It was cofunded by the European Regional Development Fund "Investing in your future" for the Thematic Network and Co-operative Research Centers ARADyAL RD16/0006/0015 and RD16/0006/0026. It was additionally supported by the Ministry of Science, Innovation in Spain (PCI2018-092930), cofunded by the European program ERA HDHL - Nutrition & the Epigenome, project Dietary Intervention in Food Allergy: Microbiome, Epigenetic and Metabolomic interactions (DIFAMEM). DR and EZ-V acknowledge funding from the Spanish Ministry of Science, Innovation and Universities (RTI2018-095166-B-I00). CU acknowledges funding from the Spanish Ministry of Economy (SAF2017-90083-R). TCB-T thanks CEU-International Doctoral School (CEINDO) for his fellowship.

Conflicts of Interest

MD Ibañez has received personal fees from Faes Farma, Merck, LETI, ROXALL, and CIRCASSIA. R Bazire reports personal fees from LETI Pharma. The remaining authors declare that they have no conflicts of interest.

Previous Presentations

The data reported here were presented as an oral communication at the EAACI 2019 Annual Congress.

References

1. Gomez de Agüero M, Ganal-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, Li H, et al. The maternal microbiota drives early postnatal innate immune development. Science. 2016;351:1296-302.
2. Wopereis H, Oozeer R, Knipping K, Belzer C, Knol J. The first thousand days - intestinal microbiology of early life: establishing a symbiosis. Pediatr Allergy Immunol. 2014;25:428-38.
3. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. Nature. 2012;486:222-7.
4. Rojo D, Méndez-García C, Raczkowska BA, Bargiela R, Moya A, Ferrer M, et al. Exploring the human microbiome from multiple perspectives: factors altering its composition and function. FEMS Microbiol Rev. 2017;41:453-78.
5. Aitoro R, Paparo L, Amoroso A, Di Costanzo M, Cosenza L, Granata V, et al. Gut Microbiota as a Target for Preventive and Therapeutic Intervention against Food Allergy. Nutrients. 2017;9:672.
6. Dong P, Feng J, Yan D, Lyu Y, Xu X. Early-life gut microbiome and cow's milk allergy- a prospective case - control 6-month follow-up study. Saudi J Biol Sci. 2018;25:875-80.
7. Lozano-Ojalvo D, Berin C, Tordesillas L. Immune Basis of Allergic Reactions to Food. J Investig Allergol Clin Immunol. 2019;29:1-14.
8. Zeiger RS, Heller S. The development and prediction of atopy in high-risk children: Follow-up at age seven years in a prospective randomized study of combined maternal and infant food allergen avoidance. J Allergy Clin Immunol. 1995;95:1179-90.
9. Zeiger RS, Heller S, Mellon MH, Halsey JF, Hamburger RN, Sampson HA. Genetic and environmental factors affecting the development of atopy through age 4 in children of atopic parents: a prospective randomized study of food allergen avoidance. Pediatr Allergy Immunol. 1992;3:110-27.
10. Pali-Schöll I, Namazy J, Jensen-Jarolim E. Allergic diseases and asthma in pregnancy, a secondary publication. World Allergy Organ J. 2017;10:10.
11. Larsen JM. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology. 2017;151:363-74.
12. Kishikawa T, Maeda Y, Nii T, Motooka D, Matsumoto Y, Matsushita M, et al. Metagenome-wide association study of gut microbiome revealed novel aetiology of rheumatoid arthritis in the Japanese population. Ann Rheum Dis. 2020;79:103-11.
13. Kim ES, Tarassishin L, Eisele C, Barre A, Nair N, Rendon A, et al. Longitudinal Changes in Fecal Calprotectin Levels Among Pregnant Women With and Without Inflammatory Bowel Disease and Their Babies. Gastroenterology. 2021;160:1118-30.e3.

14. Ruiz-Ruiz S, Sanchez-Carrillo S, Ciordia S, Mena MC, Méndez-García C, Rojo D, et al. Functional microbiome deficits associated with ageing: Chronological age threshold. Aging Cell. 2020;19:e13063.
15. Chaudhari DS, Dhotre DP, Agarwal DM, Gaike AH, Bhalerao D, Jadhav P, et al. Gut, oral and skin microbiome of Indian patrilineal families reveal perceptible association with age. Sci Rep. 2020;10:5685.

Manuscript received June 15, 2021; accepted for publication January 14, 2022.

Marina Pérez-Gordo
Instituto de Medicina Molecular Aplicada
Department of Basic Medical Sciences
Facultad de Medicina, San Pablo CEU University
28660 Madrid, Spain
E-mail: marina.perezgordo@ceu.es

