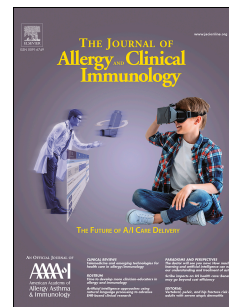


Journal Pre-proof

The maternal gut microbiome during pregnancy and offspring allergy and asthma

Yuan Gao, BSc, Ralph Nanan, Dr med. Habil., Laurence Macia, PhD, Jian Tan, PhD, Luba Sominsky, PhD, Thomas P. Quinn, MD, PhD, Martin O'Hely, PhD, Anne-Louise Ponsonby, MBBS, FAFPHM, PhD, Mimi LK. Tang, MBBS, FRACP, PhD, Fiona Collier, PhD, Deborah H. Strickland, PhD, Poshmaal Dhar, PhD, Susanne Brix, MSc, PhD, Simon Phipps, PhD, Peter D. Sly, MBBS, MD, DSc, Sarath Ranganathan, MD, PhD, Jakob Stokholm, MD, PhD, Karsten Kristiansen, PhD, Lawrence Gray, MBBS, PhD, Peter Vuillerman, MBBS, BMedSci, FRACP, PhD



PII: S0091-6749(21)01130-1

DOI: <https://doi.org/10.1016/j.jaci.2021.07.011>

Reference: YMAI 15200

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 15 June 2021

Revised Date: 19 July 2021

Accepted Date: 19 July 2021

Please cite this article as: Gao Y, Nanan R, Macia L, Tan J, Sominsky L, Quinn TP, O'Hely M, Ponsonby A-L, Tang ML, Collier F, Strickland DH, Dhar P, Brix S, Phipps S, Sly PD, Ranganathan S, Stokholm J, Kristiansen K, Gray L, Vuillerman P, The maternal gut microbiome during pregnancy and offspring allergy and asthma, *Journal of Allergy and Clinical Immunology* (2021), doi: <https://doi.org/10.1016/j.jaci.2021.07.011>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology.

The maternal gut microbiome during pregnancy and offspring allergy and asthma

Yuan Gao, BSc^{1,2,3}, Ralph Nanan, Dr med. Habil.⁴, Laurence Macia, PhD^{4,5}, Jian Tan, PhD^{4,5}, Luba Sominsky, PhD^{1,2}, Thomas P. Quinn, MD, PhD⁶, Martin O'Hely, PhD^{1,8}, Anne-Louise Ponsonby, MBBS, FAFPHM, PhD^{7,8,9}, Mimi LK Tang, MBBS, FRACP, PhD^{8,9,10}, Fiona Collier, PhD¹, Deborah H Strickland, PhD^{11,12}, Poshmaal Dhar, PhD¹, Susanne Brix, MSc, PhD¹³, Simon Phipps, PhD^{14,15,16}, Peter D. Sly MBBS, MD, DSc^{16,17}, Sarath Ranganathan, MD, PhD^{8,9,10}, Jakob Stokholm, MD, PhD^{18,19}, Karsten Kristiansen, PhD^{20,21,22}, Lawrence Gray, MBBS, PhD^{1,2*} and Peter Vuillermine, MBBS, BMedSci, FRACP, PhD^{1,2*}

* Co-senior authors

1. Institute for Physical and Mental Health and Clinical Transformation, Deakin University, Geelong, Australia
2. Child Health Research Unit, Barwon Health, Geelong, Australia
3. Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
4. The Charles Perkins Center, the University of Sydney, Sydney, Australia
5. School of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia
6. Applied Artificial Intelligence Institute, Deakin University, Geelong, Australia
7. The Florey Institute, Melbourne, Australia
8. Murdoch Children's Research Institute, Melbourne, Australia
9. University of Melbourne, Melbourne, Australia
10. Royal Children's Hospital, Melbourne, Australia
11. Telethon Institute, Perth, Western Australia
12. University of Western Australia

13. Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark.
14. QIMR Berghofer Medical Research Institute, Herston, Queensland, Australia
15. School of Biomedical Sciences, Faculty of Medicine, The University of Queensland, Queensland, Australia
16. Australian Infectious Diseases Research Centre, The University of Queensland, Queensland, Australia.
17. Children's Health and Environment Program, Child Health Research Centre, The University of Queensland, Australia.
18. COPSAC, Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, 2820 Copenhagen, Denmark.
19. Department of Pediatrics, Slagelse Hospital, 4200 Slagelse, Denmark.
20. BGI-Shenzhen, Shenzhen, China
21. China National Genebank, Shenzhen, China
22. Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Copenhagen, Denmark

Correspondence: Lawrence Gray
 The Institute for Mental and Physical Health and Clinical Translation
 (IMPACT) Barwon Health, Geelong, VIC, 3220, Australia
 e-mail: Lawrence.gray@barwonhealth.org.au

Peter Vuillermin
 The Institute for Mental and Physical Health and Clinical Translation
 (IMPACT) Barwon Health, Geelong, VIC, 3220, Australia
 e-mail: peter.vuillermin@deakin.edu.au

Phone: +61 0400071218

Conflicts of Interest

MO, ALP, MT, FC, SR and PV have a financial interest in the biotech company Prevatex Pty Ltd which seeks to develop technology regarding maternal and infant carriage of *Prevotella copri* (PCT2016905378; PCT/AU2019/050878). MT is an inventor on the patent “Methods and compositions for determining and for minimizing the likelihood of development of allergy in infants” (pending and licensed to Prevatex Limited).

Key Words: gut microbiome, fetal immunity, allergy, asthma

Abbreviations used

MAMP	Microbe-associated molecular pattern
Treg	Regulatory T cell
DC	Dendritic cell
IRF7	Interferon regulatory transcription factor 7
Th	T-helper cell
TLR	Toll-like receptor
SCFA	Short chain fatty acids
IgG	Immunoglobulin G
AIRE	Autoimmune regulator
mTOR	Mechanistic target of rapamycin
EVs	Extracellular vesicles
FMT	Fecal microbiota transplantation

Abstract

Environmental exposures during pregnancy that alter both the maternal gut microbiome and the infant's risk of allergic disease and asthma include a traditional farm environment and consumption of unpasteurized cow's milk, antibiotic use, dietary fiber and psychosocial stress. Multiple mechanisms acting in concert may underpin these associations and prime the infant to acquire immune competence and homeostasis following exposure to the extrauterine environment. Cellular and metabolic products of the maternal gut microbiome can promote the expression of microbial pattern recognition receptors, as well as thymic and bone marrow hematopoiesis relevant to regulatory immunity. At birth, transmission of maternally derived bacteria likely leverages this *in utero* programming to accelerate postnatal transition from a Th2 to Th1 and Th17 dominant immune phenotypes and maturation of regulatory immune mechanisms, which in turn reduce the child's risk of allergic disease and asthma. Although our understanding of these phenomena is rapidly evolving, the field is relatively nascent, and we are yet to translate existing knowledge into interventions that substantially reduce disease risk in humans. Here we review evidence that the maternal gut microbiome impacts the offspring's risk of allergic disease and asthma, discuss challenges and future directions for the field, and propose the hypothesis that maternal carriage of *Prevotella copri* during pregnancy decreases the offspring's risk of allergic disease via production of succinate which in turn promotes bone marrow myelopoiesis of dendritic cell precursors in the fetus.

Introduction

The modern environment, lifestyle and diet have altered the composition and function of the human gut microbiome,(1-12) which in turn plays a central role for early life immune development.(13, 14) Although the majority of research regarding the gut microbiome and allergic disease has focused on the postnatal period,(14-16) the maternal gut microbiome during pregnancy plays a key role in fetal immune programming,(17, 18) and in turn, the risk of allergic disease in the offspring.(19, 20) A range of mouse and human studies support the

putative construct shown in Figure 1: In a traditional environment, adequate exposure to environmental microbes, the absence of antibiotics, adequate dietary fiber, and lower levels of maternal psychological stress and mental illness decrease the risk of offspring allergic disease and asthma via their impacts on the mother's gut microbiome and its products. The potential underlying pathways include the alignment between maternal and infant immunity; transplacental passage of microbial metabolites and components; and possible seeding of a feto-placental microbiome. Collectively, these result in increased expression of microbe-associated molecular pattern (MAMP) recognition receptors, promotion of thymic regulatory T cells (Treg) and the establishment of dendritic cell (DC) networks in the gut, lung and other tissues. Inoculation of the infant gut with the maternal microbiota during and after birth leverages the antenatal programming to accelerate the transition from Th2 to Th1, interferon regulatory transcription factor 7 (IRF7) and Th17 dominant immunity required for pathogen clearance, and DC induction of Treg in the gut and lung required to calibrate inflammatory responses.

There are however fundamental gaps in current knowledge. Few human studies have evaluated direct microbial transfer between mother and child, nor directly examined the relationship between the maternal gut microbiome and offspring allergic disease and asthma. (19, 20) No such studies have incorporated metagenomic measures; nor have human studies demonstrated mediation pathways linking environmental factors, via maternal microbiome and infant immunity to clinical outcomes. Relatively little is known about the multitude of factors driving fetal immune development, few human studies have shown that microbiome related interventions during pregnancy alter offspring immunity, and overall, findings regarding probiotics supplementation during pregnancy are limited and conflicting. In this context, there is a clear need for improved evidence to progress and translate the potential of manipulating the maternal microbiome to prevent allergic disease and asthma.

Exposures impacting both maternal gut microbiota and offspring allergy

Farming environment

Studies in Europe, Asia, the United States and Africa have shown that early life exposure to a traditional farm environment is associated with reduced risk of allergic disease and asthma.(21-27) Although pre- and postnatal exposures were highly correlated in each of these studies, the PARSIFAL cohort was large enough to show that prenatal farm exposure may be more protective than postnatal exposure.(21)

While the ‘farm effect’ appears to relate to exposure to livestock(21, 28, 29) and a diverse range of environmental microbes,(30-33) several studies have shown that maternal consumption of unprocessed milk is also relevant.(28, 34, 35) Building on this, a recent study in mice showed that ingestion of raw versus pasteurized cow’s milk increased butyrate-producing *Clostridiales* in maternal fecal samples, decreased *Proteobacteria*, and conferred protection against IgE-mediated food allergy in the offspring.(36) The risk of *Listeria monocytogenes* infection associated with ingestion of raw milk is an important consideration, particularly in pregnancy, thus specific understanding of the underlying pathways is required to inform more targeted and safe interventions. While traditional farming is becoming uncommon in Westernized populations, the same protection from allergic disorders has been observed in rural living environments, where extended biodiversity(37) has been suggested as a mediator.

Farm children demonstrate prenatal “priming” characterized in functional studies of cord blood by higher expression of the microbial pattern recognition Toll-like receptor (TLR)7 and TLR8 receptors,(38) equipping them to respond to viral-based signals during early infancy; as well as increased Treg number and function, required to control inflammatory responses to pathogens and environmental stimuli.(39) Numerous studies have shown that both increased Th1/IFN- γ responses(40, 41), increased Treg, and enhanced levels of neutrophils(42) at birth predict decreased risk of allergic disease and asthma.(43-46) Consistent with prenatal priming, farm children demonstrate accelerated progression from Th2/eosinophil-skewed immunity at birth, which is associated with susceptibility to both infections and atopy, to a more balanced and

competent immune state characterized by robust anti-microbial responses to pathogens, and enhanced Treg function required for maintenance of immune homeostasis and control of excessive inflammation.(42) Further studies are required to evaluate the contribution of the maternal microbiome to the 'farm effect'.

Antibiotics

Antibiotics have profound and lasting effects on the gut microbiome.(47) Maternal treatment with antibiotics during pregnancy has been linked to increased eczema,(48) food allergy,(48-50) and asthma(51-53) in offspring. Evidence of a dose response between maternal treatment with antibiotics and offspring asthma in human birth cohort studies(54) and experimental models(55) strengthens the case for causality. Antibiotic treatment during pregnancy may influence both the maternal and infant gut microbiome in mice,(56) so it is difficult to delineate the relative importance of the pre and postnatal periods. It is also impossible to exclude confounding by indication - in particular antibiotic treatment of maternal respiratory infections which may relate to maternal asthma, and thus shared maternal/infant genetic risk. From large register-based studies, where it is also possible to evaluate associations with antibiotic exposure to the mother around the pregnancy period(57) and by applying siblings design(58), evidence of antibiotic use in pregnancy as a causal link to later disease disappears. Instead, these studies suggest that a susceptibility to infections may be the factor inherited, as the mother's use of antibiotics outside the pregnancy period was just as important a risk factor for asthma in the child, and higher risk of disease was also found in siblings unexposed to maternal antibiotics during pregnancy.

Diet

Although the relationship between maternal diet and offspring allergic disease remains controversial, recent work has added to the evidence that a maternal diet high in vegetables is associated with a reduced risk of offspring allergic disease in humans.(59) Diet has an important impact on gut microbiota composition and metabolic function.(9) Ecological studies have consistently shown associations between a pre-agrarian, hunter-gatherer diet and lifestyle and increased diversity of the gut microbiota, and increased production of specific microbial

metabolites, in particular, short chain fatty acids (SCFAs).(1, 2, 10) In each of these studies, the hunter-gatherer communities had a diet high in plant derived fibers,(60) which have a central role in shaping the composition and metabolic activity of the gut microbiome.(11) A maternal diet high in fiber is associated with increased diversity and richness of gut microbiota during pregnancy, with increases in specific taxa including *Holdemania*, *Roseburia*, and *Lachnospira* and *Coproccoccus*.(61-63) Studies in mice have shown that a diet low in fiber leads to intergenerational depletion in the diversity of gut microbiota which only partially recovers following reinstitution of a high fiber diet.(64) This suggests that the low fiber diet which is now endemic in many parts of the world is contributing to intergenerational loss of our ‘ancestral’ gut microbes. Anaerobic bacteria in the gut ferment fiber to produce SCFAs, which have potent local and systemic immune effects(65) and can cross the placenta. In mice, a maternal diet high in fiber attenuates inflammatory airway responses to ovalbumin challenge in the offspring.(66) Although the underlying mechanisms are not fully characterized, SCFA induction of Treg may be relevant(67) and human studies investigating maternal diet-by-microbiome pathways to enhanced infant immune competence required.

Psychosocial stress and mental illness

Maternal psychosocial stress and mental illness during pregnancy are associated with increased risk of allergy and asthma in the offspring.(68-70) Stress-induced release of glucocorticoids has been suggested to mediate these effects,(71) but the impact of psychological stress and anxiety on the maternal gut microbiome may also be relevant (12, 72-74). In support of this, animal studies have demonstrated that maternal psychosocial stress before and during pregnancy may alter both the mothers’ and infants’ intestinal microbiota, in part mediating the effects of maternal stress on immune development of the offspring.(75) Related studies are needed in humans.

Potential Mechanisms

Maternal-fetal immune alignment

Preparing the fetal immune system *in utero* for the extrauterine environment is germane for survival, with maternally conferred immune protection against environmental pathogens representing a key defense mechanism. In this context, maternal transfer of humoral immunity, specifically of maternal immunoglobulin G (IgG) through an active mechanism enabled through neonatal Fc receptors, has been well established.(76) Through this process serum IgG levels of the term newborn are equal to or even exceed maternal levels. Of note, maternal IgG transfer of gut microbial components to the offspring during gestation also plays a crucial role in fetal innate immune development in mice.(18) Postpartum, transfer of maternal immunoglobulins continues through lactation, but these antibodies confer mucosal immunity only.(77)

In contrast, for cellular immunity it has been a long-held view that the fetus holds an immune privileged status, keeping the fetal and maternal immune cells separate, allowing physiologic development of the semi-allogenic fetus without triggering inflammatory responses.(78) Immune tolerance towards the fetus is upheld by a series of highly effective mechanisms, with the placenta providing a barrier preventing immune rejection and/or trafficking of immune cells across the maternal-fetal interface.(79) The latter concept has been challenged by studies providing compelling evidence that traces of maternal cells can be found in fetal organs including the thymus(80) and vice versa.(81) This early exchange seems to shape the offspring's immune response and can in part explain the better acceptance of maternal versus paternal organ transplants.(82)

There is also evidence for alignment of maternal and fetal lymphocytes, specifically of Treg cells. This alignment is likely linked to soluble factors that cross the placenta from the mother to the fetus and effectively influence the size of the fetal Treg cell pool(83, 84) and metabolites of the maternal gut microbiome are likely candidates.(85)

Bacterial metabolites

The SCFAs butyrate, propionate and acetate are by-products of dietary fiber fermentation by gut bacteria.(60) In mouse models, maternal SCFAs attenuate inflammatory airway response in offspring.(66) Although the underlying mechanism is unknown, SCFAs have potent immunomodulatory properties including induction of Treg from naïve T cells via dendritic cells either through epigenetic changes by inhibiting histone deacetylases,(66, 86, 87) or through the activation of specific G-protein coupled receptor GPR43.(88) Recently, acetate has been identified as an important factor in fetal thymic development, linked to the expression of the autoimmune regulator (AIRE), which is known to contribute to Treg cell generation.(89) Succinate is another immune active microbial metabolite which is increased in animals fed a high fiber and fat diet(90). Succinate binds GPR91, stimulating dendritic cells (DC) and enhancing DC-mediated T cell induction and cytokine production.(91) Further studies are needed to investigate the impact of these metabolites of the maternal microbiome on fetal immune development and offspring allergic disease.

Bacterial components

In mice, repeated administration of the bacterial lysate OM-85 during pregnancy, attenuates aeroallergen induced asthma-like responses in the offspring.(92) This finding is consistent with earlier work showing that maternal oral intake of *Acinetobacter lwoffii* F78, derived from farm (barn) dust, during pregnancy attenuated aeroallergen induced inflammation in the offspring, and that this process was TLR-dependent.(93) The offspring of mothers treated with OM-85 during pregnancy demonstrate increased capacity for expansion and functional activation of Tregs in the airway mucosa in response to aeroallergen challenge.(92) This enhanced regulatory capacity is underpinned by a dramatic expansion of bone marrow myeloid progenitors during late pregnancy; which in turn supply the establishment of tissue dendritic cell networks that mediate local immune surveillance and homeostasis.(92) Subsequent studies have shown that maternal OM-85 treatment is associated with transcriptional activation of cellular cholesterol biosynthesis pathways (mediated via the mevalonate pathway) and expansion of myeloid progenitors (myelopoiesis) and downstream conventional dendritic cells displaying enhanced

functional maturation within fetal bone marrow.(94) The establishment of dendritic cell networks in late gestation can be considered a form of immune training – a process in which innate immune cells exhibit a sustained state of enhanced functionality. Recently, it has also been demonstrated that treatment of pregnant mice with OM-85 during pregnancy protects offspring against mouse-adapted rhinovirus, reducing cellular inflammation and clinical severity of lung disease.(95) Studies needed to determine whether maternal gut microbiota and their products promote fetal immune development via the same pathways.

Bacterial extracellular vesicles

Like other cells, gut bacteria produce extracellular vesicles (EVs), which are blebs of plasma membrane carrying a cargo of intracellular products such as proteins, nucleic acids, and lipids.(96) There is growing interest in the role of EVs in mediating the immune effects of gut bacteria. For example, the activation of TLR2 by *Bacteroides fragilis* has been shown to be mediated by the release of EVs.(97) In postnatal mouse studies, the administration of *Lactobacillus plantarum* EVs prevented the development of atopic dermatitis,(98) while the administration of EVs from *Bifidobacterium longum* prevented food allergy.(99) Given that EVs are small structures that can be actively transported from the mother to the fetus(100), studies are needed to evaluate whether EVs produced by maternal gut microbiota impact fetal immune development.

In utero bacterial colonization

The presence of bacteria in sites including placenta, amniotic fluid, and meconium, was reported in 2011,(101, 102) and has since been the subject of intense debate. Subsequent studies have identified bacteria in placenta,(103-107) amniotic fluid,(108-113) and meconium(105, 106, 114-116); and one study found that maternal consumption of probiotics and contact with furry pets during pregnancy may each influence the microbiota present in meconium.(117)

Ascension and translocation through the choriodecidual barriers is one potential mechanism of *in utero* bacterial colonization.(104, 110, 118) Alternatively, microbes derived from the

maternal intestine and oral cavity may be translocated by hematogenous spread to the placenta during implantation,(18, 104) due to higher intercellular junctional permeability and/or dendritic cell transport.(119) Translocation of bacteria to the maternal circulation, via the epithelial gaps of the intestine and the oral mucosa, is enhanced during pregnancy and this enables the transfer of low numbers of bacteria to possibly seed the placenta.(104, 120-122)

Although some studies suggest that the presence of bacterial DNA in pregnancy tissues may relate to contamination of samples or reagents,(106, 107, 123) it remains a possibility that a fetoplacental microbiome plays a role in fetal immune development. The meconium microbiota was found to reflect microbial composition of the amniotic fluid,(124) suggesting the fetal intestine could become colonized via ingestion of small quantities of amniotic fluid during the antenatal period and thus the meconium microbiome could be a proxy for the fetoplacental microbiome. Of relevance, a study from the Canadian CHILd cohort suggests that the metabolic diversity of meconium collected at birth predicts both the infant's postnatal gut microbiota and risk of allergic sensitization at 1 year.(125) Moreover, a recent study found live bacterial strains including *Staphylococcus* and *Lactobacillus* in fetal tissue, which induced in vitro activation of memory T cells in fetal mesenteric lymph nodes, supporting a role of microbial exposure in fetal immune programming.(126)

Vertical transmission of gut microbiota at birth

In infants delivered vaginally, the maternal gut microbiome is the largest donor of the infant-acquired strains(127, 128) while the maternal vaginal transfer appears of minor importance.(129) Strain-level analyses of mother–infant pairs revealed that 50% of the microbial species in the infant gut on the day of delivery were transmitted from the mother's microbiome, and this fraction was relatively stable over the next 4 months.(127) The largest contribution at birth was from the mothers' gut, accounting for 22%, followed by the vagina (16%), the oral cavity (7%), and the skin (5%).(127) Moreover, the maternal gut microbial strains were more persistent in the infant's gut and more stably detected than those obtained from other maternal or environmental sources.(127, 128, 130)

It is therefore unsurprising that mode of birth profoundly influences the infant's gut microbiome (130-135). Although the link between mode of birth and allergic disease remains controversial, a recently study found that increased risk of asthma following cesarean delivery was only apparent in children who retained a 'cesarean section signature' in the gut microbiome to age 1 year.(136) Strain-level analyses directly implicate the interruption of maternal-infant transmission in this effect.(127, 128, 135, 137) Interventions proposed to offset the alteration in gut microbiota induced by cesarean section include dietary supplementation with a mix of probiotics during pregnancy and to the infants,(138) and postnatally, orally delivered fecal microbiota transplantation (FMT) from their mothers.(139) Both techniques appear to ameliorate birth mode-associated differences in infant intestinal microbiota but there is currently no evidence that either reduces the baby's risk of allergic disease or asthma.

Direct links between maternal gut microbiota and offspring allergy in humans

As far as we are aware, only two published studies have directly investigated the relationship between the maternal gut microbiota and offspring allergic disease in humans. A small study (n=60) from 2012 used culture-based techniques to show that higher total aerobes and *Enterococci* in maternal fecal samples predicted increased risk of infant wheeze.(20) More recently, we used 16S gene amplicon sequencing to demonstrate that maternal carriage of *Prevotella copri* during pregnancy predicts decreased IgE-mediated food allergy in the offspring.(19) Importantly, maternal rather than infant carriage drove this association. *Prevotella* carriage is virtually ubiquitous in a pre-agrarian, hunter-gatherer populations(1, 2, 10) and the genus is among the so-called 'ancestral microbes' present in the human gut at least as far back as the Neanderthal period,(8, 140) and which are now disappearing in westernized populations.(1-8) *P. copri* is the predominant *Prevotella* species within the human gut microbiome. Our study showed that reduced household size and the recent exposure to antibiotics were associated with decreased maternal carriage of *P. copri*.(19) The link between household size and *P. copri* carriage is consistent with previous studies,(141) and intriguing given the well-known, yet still unexplained, association between larger household size and decreased allergic disease.(142) *P.copri* is an important producer of succinate,(143) which can

stimulate function and migration of dendritic cells (DC).(144) Previous studies have shown that the metabolic products of the gut microbiome stimulate bone marrow hemopoiesis of DC precursors, which then migrate to peripheral tissues including the lung, attenuating the features of allergic airways disease.(145) Considering the mounting evidence regarding the role of DC hemopoiesis in late pregnancy to prevention of allergic disease,(92-94) we hypothesize that maternal carriage of *P. copri*, via production of succinate, promotes the establishment of fetal DC networks, conveying protection against allergic disease and asthma (Figure 2).

Probiotic supplementation during pregnancy

Recent meta-analyses have found no consistent beneficial effects from probiotic supplementation (either prenatal, postnatal or both) for prevention of eczema(146) or asthma.(147) These inconsistencies may be due to differences in the study populations, the probiotic strains, using a single strain rather than a complex mixture, and administration protocols. Although several trials found that combined maternal and infant probiotic supplementation with various strains of *Lactobacillus* reduced the risk of allergy,(148) others found no benefit(149, 150); and the only trial of *Lactobacillus* in pregnancy alone was negative.(151) Potential benefits may be modified by mode of delivery. One trial randomized high risk mothers to receive either a probiotic mixture (*L. rhamnosus* GG ATCC 53103 5, *L. rhamnosus* LC705 DSM 7061, *Bifidobacterium breve* Bb99 DSM 13692, and *Propionibacterium freudenreichii* ssp. shermanii JS DSM 7076) or placebo twice daily starting from 36 weeks of gestation.(152) Their infants then received the same probiotic combination mixed with galacto-oligosaccharides once daily from birth until 6 months. Treatment with another probiotic mixture was also associated with a reduced risk of allergen sensitization and eczema among children born via cesarean section but not among those born vaginally. Another trial found that maternal ingestion of probiotic milk containing *Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* Bb12, or *L. rhamnosus* in pregnancy was associated with a slightly reduced relative risk of atopic eczema at 6 months and allergic rhinitis between 18 and 36 months compared with no consumption during pregnancy.(153)

Importantly, to date there have been no trials of supplementation with Gram-negative organisms, which may be relevant given that oral administration of the Gram-negative *Acinetobacter lwoffii* F78 in mice resulted in decreased allergy in the offspring.(93) This benefit was TLR-dependent, suggesting that lipopolysaccharide in the cell membrane of Gram-negative organisms may be important. Another consideration is that, in general, probiotic strains are specifically chosen that do not colonize the bowel, i.e. they do not live permanently and multiply in the gastrointestinal tract. It is possible that active growth and multiplication is required to produce the metabolites that influence fetal and neonatal immune development, with subsequent reduction in offspring allergic disease.

Future directions

Correcting the absence of deep whole metagenome sequencing data from human prebirth cohorts incorporating robust allergy and asthma outcomes is a priority. In contrast to 16S rRNA gene amplicon, deep metagenomic sequencing provides sufficient resolution at the species and strain level to infer the functional capacity and nutrient interactions of a given bacterium.(154) Estimation of absolute abundances of microbiota may also provide more accurate information than currently used measures of relative abundance.(155) In principle, the integration of metagenomics, downstream measures of microbiome activity including metabolomics, transcriptomics, proteomics and lipidomics with various immune measures has significant promise. However, multi-omic data generated using separate assays over the course of pre- and postnatal life are both multi-modal and temporal in nature. As such, their analyses and interpretation pose formidable challenges, and methodological frameworks built specifically for modelling time-varying multi-omic data are required.(156) The compositional nature of microbiome data also has important implications.(157) This is generally addressed through various forms of pre-processing steps; the choice of which can substantially impact the results(158) making quantitative metagenomics a preferable strategy, whenever possible.

Robust consideration of confounding bias and mediation pathways also poses an important, but neglected, challenge for the field. Microbiome data are highly dimensioned, and the

determinants of specific components/metrics are largely unknown, thus *a priori* identification of common causes of exposures and outcomes (confounders) is largely speculative. One approach to this dilemma is to apply the so-called disjunctive cause criterion,(159) which has an elegant proof, and removes the need for prior knowledge of common causes. Others argue that a strictly *a priori*- defined approach to estimating causal effects using highly dimensioned data risks missing important signals and that one should therefore conduct multiple analyses aimed to build up layers of causal features and exclude non-casual explanations such as bias, chance, or reverse causation.(160)

Whichever approach is taken to pre-processing and analysis of human multi-omics data, it is crucial to formulate clear research questions, and to then choose methods that are appropriate. This is a highly complex task requiring clinical, biological, bioinformatic, biostatistical and epidemiological input. Reporting from human studies should include effect sizes both with and without adjustment along with a clear description of the causal framework that underlies the model(s) that have been applied, and the sensitivity of the findings to key assumptions. Importantly, the risk of false discoveries in highly dimensioned data must be controlled for and replication is crucial.

Progressing from correlation in human microbiome studies to causation and effective interventions requires mechanistic insights.(161) Preclinical studies have generally involved the transplantation of bacteria from humans with and without disease into germ-free mice and comparative analysis of pathological outcomes. While this approach has merit, it is of concern that the vast majority of published studies using this approach have found that transplantation of a given human derived microbe results in altered expression of the relevant disease phenotype in mice, which is implausible, and a more rigorous approach to inferring causality is required.(162)

Conclusions

The maternal gut microbiome plays a fundamental role in priming the infant's developing immune system to acquire immune competence and regulation following exposure to the extrauterine environment. Evidence from a diverse range of studies suggests that loss of our ancestral gut microbes and altered metabolic activity of the maternal microbiome may be contributing to the high rate of allergic disease and asthma in the modern environment. While rapid progress in omics technology has created tantalizing opportunities to progress current knowledge regarding interplay between the microbiome and early life immunity, analysis and interpretation of such complex data brings formidable challenges. In this context, transdisciplinary collaboration is needed to generate strong and clearly defined research questions, fit-for-purpose analysis plans, and iterative human and animal research to identify mechanisms and confirm causality.

References

1. Schnorr SL, Candela M, Rampelli S, Centanni M, Consolandi C, Basaglia G, et al. Gut microbiome of the Hadza hunter-gatherers. *Nature Communications*. 2014;5(1):3654.
2. Martínez I, Stegen James C, Maldonado-Gómez Maria X, Eren AM, Siba Peter M, Greenhill Andrew R, et al. The Gut Microbiota of Rural Papua New Guineans: Composition, Diversity Patterns, and Ecological Processes. *Cell Reports*. 2015;11(4):527-38.
3. Ayeni FA, Biagi E, Rampelli S, Fiori J, Soverini M, Audu HJ, et al. Infant and Adult Gut Microbiome and Metabolome in Rural Bassa and Urban Settlers from Nigeria. *Cell Rep*. 2018;23(10):3056-67.
4. Girard C, Tromas N, Amyot M, Shapiro BJ. Gut Microbiome of the Canadian Arctic Inuit. *mSphere*. 2017;2(1).
5. Jha AR, Davenport ER, Gautam Y, Bhandari D, Tandukar S, Ng KM, et al. Gut microbiome transition across a lifestyle gradient in Himalaya. *PLoS Biol*. 2018;16(11):e2005396.
6. Martinez I, Stegen JC, Maldonado-Gomez MX, Eren AM, Siba PM, Greenhill AR, et al. The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes. *Cell reports*. 2015;11(4):527-38.
7. Obregon-Tito AJ, Tito RY, Metcalf J, Sankaranarayanan K, Clemente JC, Ursell LK, et al. Subsistence strategies in traditional societies distinguish gut microbiomes. *Nat Commun*. 2015;6:6505.
8. Rampelli S, Turrone S, Mallol C, Hernandez C, Galvan B, Sistiaga A, et al. Components of a Neanderthal gut microbiome recovered from fecal sediments from El Salt. *Commun Biol*. 2021;4(1):169.
9. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*. 2014;505(7484):559-63.
10. 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(7402):222.
11. De Filippo C, Di Paola M, Ramazzotti M, Albanese D, Pieraccini G, Banci E, et al. Diet, Environments, and Gut Microbiota. A Preliminary Investigation in Children Living in Rural and Urban Burkina Faso and Italy. *Frontiers in Microbiology*. 2017;8:1979.
12. Hechler C, Borewicz K, Beijers R, Saccenti E, Riksen-Walraven M, Smidt H, et al. Association between Psychosocial Stress and Fecal Microbiota in Pregnant Women. *Scientific Reports*. 2019;9(1):4463.
13. Chung H, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, et al. Gut immune maturation depends on colonization with a host-specific microbiota. *Cell*. 2012;149(7):1578-93.
14. Ruotula T, de Goffau MC, Nieminen JK, Honkanen J, Siljander H, Hämäläinen A-M, et al. Maturation of Gut Microbiota and Circulating Regulatory T Cells and Development of IgE Sensitization in Early Life. *Frontiers in Immunology*. 2019;10:2494.
15. Depner M, Taft DH, Kirjavainen PV, Kalanetra KM, Karvonen AM, Peschel S, et al. Maturation of the gut microbiome during the first year of life contributes to the protective farm effect on childhood asthma. *Nat Med*. 2020;26(11):1766-75.

16. Feehley T, Plunkett CH, Bao R, Choi Hong SM, Culleen E, Belda-Ferre P, et al. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nature Medicine*. 2019;25(3):448-53.
17. Jašarević E, Bale TL. Prenatal and postnatal contributions of the maternal microbiome on offspring programming. *Frontiers in Neuroendocrinology*. 2019;55:100797.
18. Gomez de Agüero M, Ganai-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, Li H, et al. The maternal microbiota drives early postnatal innate immune development. *Science (New York, NY)*. 2016;351(6279):1296-302.
19. Institute tJCV, Group tBI, Vuillermine PJ, O'Hely M, Collier F, Allen KJ, et al. Maternal carriage of *Prevotella* during pregnancy associates with protection against food allergy in the offspring. *Nature Communications*. 2020;11(1):1452.
20. Lange NE, Celedón JC, Forno E, Ly NP, Onderdonk A, Bry L, et al. Maternal intestinal flora and wheeze in early childhood. *Clinical & Experimental Allergy*. 2012;42(6):901-8.
21. Douwes J, Cheng S, Travier N, Cohet C, Niesink A, McKenzie J, et al. Farm exposure in utero may protect against asthma, hay fever and eczema. *European Respiratory Journal*. 2008;32(3):603-11.
22. Ege M, Bieli C, Frei R, Vanstrien R, Riedler J, Ublagger E, et al. Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *Journal of Allergy and Clinical Immunology*. 2006;117(4):817-23.
23. Genuneit J, Büchele G, Waser M, Kovacs K, Debinska A, Boznanski A, et al. The GABRIEL Advanced Surveys: study design, participation and evaluation of bias. *Paediatric and Perinatal Epidemiology*. 2011;25(5):436-47.
24. Yu J, Liu X, Li Y, Meng S, Wu F, Yan B, et al. Maternal exposure to farming environment protects offspring against allergic diseases by modulating the neonatal TLR-Tregs-Th axis. *Clinical and Translational Allergy*. 2018;8(1):34.
25. Dong G-H, Ding H-L, Ma Y-N, Jin J, Cao Y, Zhao Y-D, et al. Asthma and asthma-related symptoms in 16 789 Chinese children in relation to pet keeping and parental atopy. *Journal of Investigational Allergology & Clinical Immunology*. 2008;18(3):207-13.
26. Stein MM, Hrusch CL, Gozdz J, Igartua C, Pivniouk V, Murray SE, et al. Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children. *The New England journal of medicine*. 2016;375(5):411-21.
27. Levin ME, Botha M, Basera W, Facey-Thomas HE, Gaunt B, Gray CL, et al. Environmental factors associated with allergy in urban and rural children from the South African Food Allergy (SAFFA) cohort. *Journal of Allergy and Clinical Immunology*. 2020;145(1):415-26.
28. Riedler J, Braun-Fahrlander C, Eder W, Schreuer M, Waser M, Maisch S, et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet (London, England)*. 2001;358(9288):1129-33.
29. Illi S, Depner M, Genuneit J, Horak E, Loss G, Strunz-Lehner C, et al. Protection from childhood asthma and allergy in Alpine farm environments—the GABRIEL Advanced Studies. *Journal of Allergy and Clinical Immunology*. 2012;129(6):1470-7.e6.
30. Ege MJ, Mayer M, Normand A-C, Genuneit J, Cookson WOCM, Braun-Fahrlander C, et al. Exposure to Environmental Microorganisms and Childhood Asthma. *New England Journal of Medicine*. 2011;364(8):701-9.

31. Steiman CA, Evans MD, Lee KE, Lasarev MR, Gangnon RE, Olson BF, et al. Patterns of farm exposure are associated with reduced incidence of atopic dermatitis in early life. *Journal of Allergy and Clinical Immunology*. 2020;146(6):1379-86.e6.
32. Schram D, Doekes G, Boeve M, Douwes J, Riedler J, Ublagger E, et al. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children - the PARSIFAL Study. *Allergy*. 2005;60(5):611-8.
33. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental Exposure to Endotoxin and Its Relation to Asthma in School-Age Children. *New England Journal of Medicine*. 2002;347(12):869-77.
34. Waser M, Michels KB, Bieli C, Flöistrup H, Pershagen G, von Mutius E, et al. Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe. *Clinical & Experimental Allergy*. 2007;37(5):661-70.
35. Brick T, Schober Y, Böcking C, Pekkanen J, Genuneit J, Loss G, et al. ω -3 fatty acids contribute to the asthma-protective effect of unprocessed cow's milk. *Journal of Allergy and Clinical Immunology*. 2016;137(6):1699-706.e13.
36. Abbring S, Engen PA, Naqib A, Green SJ, Garssen J, Keshavarzian A, et al. Raw Milk-Induced Protection against Food Allergic Symptoms in Mice Is Accompanied by Shifts in Microbial Community Structure. *International Journal of Molecular Sciences*. 2021;22(7):3417.
37. von Hertzen L, Hanski I, Haahtela T. Natural immunity. Biodiversity loss and inflammatory diseases are two global megatrends that might be related. *EMBO Rep*. 2011;12(11):1089-93.
38. Ober C, Sperling AI, von Mutius E, Vercelli D. Immune development and environment: lessons from Amish and Hutterite children. *Curr Opin Immunol*. 2017;48:51-60.
39. Schaub B, Liu J, Höppler S, Schleich I, Huehn J, Olek S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. *Journal of Allergy and Clinical Immunology*. 2009;123(4):774-82.e5.
40. Tang MLK, Kemp AS, Hill DJ, Thorburn J. Reduced interferon- γ secretion in neonates and subsequent atopy. *The Lancet*. 1994;344(8928):983-5.
41. Prescott SL, King B, Strong TL, Holt PG. The value of perinatal immune responses in predicting allergic disease at 6 years of age. *Allergy*. 2003;58(11):1187-94.
42. Ober C, Sperling AI, von Mutius E, Vercelli D. Immune development and environment: lessons from Amish and Hutterite children. *Current opinion in immunology*. 2017;48:51-60.
43. Hinz D, Bauer M, Roder S, Olek S, Huehn J, Sack U, et al. Cord blood Tregs with stable FOXP3 expression are influenced by prenatal environment and associated with atopic dermatitis at the age of one year. *Allergy*. 2012;67(3):380-9.
44. Zhang Y, Collier F, Naselli G, Saffery R, Tang ML, Allen KJ, et al. Cord blood monocyte-derived inflammatory cytokines suppress IL-2 and induce nonclassic "T_H-2-type" immunity associated with development of food allergy. *Science Translational Medicine*. 2016;8(321):321ra8-ra8.
45. Collier F, Ponsonby AL, O'Hely M, Tang MLK, Saffery R, Molloy J, et al. Naïve regulatory T cells in infancy: Associations with perinatal factors and development of food allergy. *Allergy*. 2019;74(9):1760-8.

46. Gray LEK, Ponsonby AL, Collier F, O'Hely M, Sly PD, Ranganathan S, et al. Deserters on the atopic march: Risk factors, immune profile and clinical outcomes of food sensitized-tolerant infants. *Allergy*. 2020;75(6):1404-13.
47. Francino MP. Antibiotics and the Human Gut Microbiome: Dysbioses and Accumulation of Resistances. *Frontiers in Microbiology*. 2016;6.
48. Gao X, Yan Y, Zeng G, Sha T, Liu S, He Q, et al. Influence of prenatal and early-life exposures on food allergy and eczema in infancy: a birth cohort study. *BMC Pediatrics*. 2019;19(1):239.
49. Metzler S, Frei R, Schmaußer - Hechfellner E, von Mutius E, Pekkanen J, Karvonen AM, et al. Association between antibiotic treatment during pregnancy and infancy and the development of allergic diseases. *Pediatric Allergy and Immunology*. 2019;30(4):423-33.
50. Timm S, Schlünssen V, Olsen J, Ramlau-Hansen CH. Prenatal antibiotics and atopic dermatitis among 18-month-old children in the Danish National Birth Cohort. *Clinical & Experimental Allergy*. 2017;47(7):929-36.
51. Metsälä J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. *Clinical & Experimental Allergy*. 2015;45(1):137-45.
52. Lapin B, Piorkowski J, Ownby D, Freels S, Chavez N, Hernandez E, et al. Relationship between prenatal antibiotic use and asthma in at-risk children. *Annals of Allergy, Asthma & Immunology*. 2015;114(3):203-7.
53. Mulder B, Pouwels KB, Schuiling-Veninga CCM, Bos HJ, de Vries TW, Jick SS, et al. Antibiotic use during pregnancy and asthma in preschool children: the influence of confounding. *Clinical & Experimental Allergy*. 2016;46(9):1214-26.
54. Loewen K, Monchka B, Mahmud SM, 't Jong G, Azad MB. Prenatal antibiotic exposure and childhood asthma: a population-based study. *European Respiratory Journal*. 2018;52(1):1702070.
55. Alhasan MM, Cait AM, Heimesaat MM, Blaut M, Klopfleisch R, Wedel A, et al. Antibiotic use during pregnancy increases offspring asthma severity in a dose - dependent manner. *Allergy*. 2020;75(8):1979-90.
56. Gonzalez-Perez G, Hicks AL, Tekieli TM, Radens CM, Williams BL, Lamoussé-Smith ESN. Maternal Antibiotic Treatment Impacts Development of the Neonatal Intestinal Microbiome and Antiviral Immunity. *The Journal of Immunology*. 2016;196(9):3768-79.
57. Stokholm J, Sevelsted A, Bonnelykke K, Bisgaard H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. *The Lancet Respiratory medicine*. 2014;2(8):631-7.
58. Ortqvist AK, Lundholm C, Kieler H, Ludvigsson JF, Fall T, Ye W, et al. Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis. *BMJ*. 2014;349:g6979.
59. Venter C, Palumbo MP, Glueck DH, Sauder KA, O'Mahony L, Fleischer DM, et al. The Maternal Diet Index in Pregnancy is associated with offspring allergic diseases: The Healthy Start Study. *Allergy*. 2021.
60. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The Role of Short-Chain Fatty Acids in Health and Disease. *Advances in Immunology*. 121: Elsevier; 2014. p. 91-119.

61. Barrett HL, Gomez-Arango LF, Wilkinson SA, McIntyre HD, Callaway LK, Morrison M, et al. A Vegetarian Diet Is a Major Determinant of Gut Microbiota Composition in Early Pregnancy. *Nutrients*. 2018;10(7):890.
62. Gomez-Arango LF, Barrett HL, Wilkinson SA, Callaway LK, McIntyre HD, Morrison M, et al. Low dietary fiber intake increases *Collinsella* abundance in the gut microbiota of overweight and obese pregnant women. *Gut Microbes*. 2018;9(3):189-201.
63. R  yti   H, Mekkala K, Vahlberg T, Laitinen K. Dietary intake of fat and fibre according to reference values relates to higher gut microbiota richness in overweight pregnant women. *Br J Nutr*. 2017;118(5):343-52.
64. Sonnenburg ED, Smits SA, Tikhonov M, Higginbottom SK, Wingreen NS, Sonnenburg JL. Diet-induced extinctions in the gut microbiota compound over generations. *Nature*. 2016;529(7585):212-5.
65. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*. 2009;461(7268):1282-6.
66. Thorburn AN, McKenzie CI, Shen S, Stanley D, Macia L, Mason LJ, et al. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nature communications*. 2015;6:7320.
67. Nakajima A, Kaga N, Nakanishi Y, Ohno H, Miyamoto J, Kimura I, et al. Maternal High Fiber Diet during Pregnancy and Lactation Influences Regulatory T Cell Differentiation in Offspring in Mice. *The Journal of Immunology*. 2017;199(10):3516-24.
68. van de Loo KFE, van Gelder MMHJ, Roukema J, Roeleveld N, Merkus PJFM, Verhaak CM. Prenatal maternal psychological stress and childhood asthma and wheezing: a meta-analysis. *European Respiratory Journal*. 2016;47(1):133-46.
69. Andersson NW, Hansen MV, Larsen AD, Hougaard KS, Kolstad HA, Schl  nssen V. Prenatal maternal stress and atopic diseases in the child: a systematic review of observational human studies. *Allergy*. 2016;71(1):15-26.
70. Flanigan C, Sheikh A, DunnGalvin A, Brew BK, Almqvist C, Nwaru BI. Prenatal maternal psychosocial stress and offspring's asthma and allergic disease: A systematic review and meta-analysis. *Clinical & Experimental Allergy*. 2018;48(4):403-14.
71. Veru F, Laplante DP, Luheshi G, King S. Prenatal maternal stress exposure and immune function in the offspring. *Stress*. 2014;17(2):133-48.
72. Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nature Reviews Gastroenterology & Hepatology*. 2009;6(5):306-14.
73. Foster JA, Lyte M, Meyer E, Cryan JF. Gut Microbiota and Brain Function: An Evolving Field in Neuroscience: Table 1. *International Journal of Neuropsychopharmacology*. 2016;19(5):pyv114.
74. Dinan TG, Cryan JF. Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration: Microbiota-gut-brain axis across the lifespan. *The Journal of Physiology*. 2017;595(2):489-503.
75. Ja  arevi   E, Rodgers AB, Bale TL. A novel role for maternal stress and microbial transmission in early life programming and neurodevelopment. *Neurobiology of Stress*. 2015;1:81-8.
76. Patel DD, Bussell JB. Neonatal Fc receptor in human immunity: Function and role in therapeutic intervention. *Journal of Allergy and Clinical Immunology*. 2020;146(3):467-78.

77. Carr LE, Virmani MD, Rosa F, Munblit D, Matazel KS, Elolimy AA, et al. Role of Human Milk Bioactives on Infants' Gut and Immune Health. *Frontiers in Immunology*. 2021;12:604080.
78. Kanellopoulos-Langevin C, Caucheteux SM, Verbeke P, Ojcius DM. Tolerance of the fetus by the maternal immune system: role of inflammatory mediators at the feto-maternal interface. *Reproductive Biology and Endocrinology*. 2003;1(1):121.
79. Tersigni C, Meli F, Neri C, Iacoangeli A, Franco R, Lanzone A, et al. Role of Human Leukocyte Antigens at the Feto-Maternal Interface in Normal and Pathological Pregnancy: An Update. *International Journal of Molecular Sciences*. 2020;21(13):4756.
80. Mold JE, Michaelsson J, Burt TD, Muench MO, Beckerman KP, Busch MP, et al. Maternal Alloantigens Promote the Development of Tolerogenic Fetal Regulatory T Cells in Utero. *Science*. 2008;322(5907):1562-5.
81. Boddy AM, Fortunato A, Wilson Sayres M, Aktipis A. Fetal microchimerism and maternal health: A review and evolutionary analysis of cooperation and conflict beyond the womb. *BioEssays*. 2015;37(10):1106-18.
82. Burlingham WJ, Grailer AP, Heisey DM, Claas FHJ, Norman D, Mohanakumar T, et al. The Effect of Tolerance to Noninherited Maternal HLA Antigens on the Survival of Renal Transplants from Sibling Donors. *New England Journal of Medicine*. 1998;339(23):1657-64.
83. Santner-Nanan B, Straubinger K, Hsu P, Parnell G, Tang B, Xu B, et al. Fetal–Maternal Alignment of Regulatory T Cells Correlates with IL-10 and Bcl-2 Upregulation in Pregnancy. *The Journal of Immunology*. 2013;191(1):145-53.
84. Bordon Y. Bacterial metabolites shape neonatal immune system. *Nature Reviews Immunology*. 2019;19(9):537-.
85. Hu M, Eviston D, Hsu P, Marino E, Chidgey A, Santner-Nanan B, et al. Decreased maternal serum acetate and impaired fetal thymic and regulatory T cell development in preeclampsia. *Nat Commun*. 2019;10(1):3031.
86. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, Deroos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504(7480):451.
87. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013;504(7480):446.
88. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. 2013;341(6145):569-73.
89. Group TBI, Hu M, Eviston D, Hsu P, Mariño E, Chidgey A, et al. Decreased maternal serum acetate and impaired fetal thymic and regulatory T cell development in preeclampsia. *Nature Communications*. 2019;10(1):3031.
90. Zhong Y, Marungruang N, Fåk F, Nyman M. Effects of two whole-grain barley varieties on caecal SCFA, gut microbiota and plasma inflammatory markers in rats consuming low-and high-fat diets. *British Journal of Nutrition*. 2015;113(10):1558-70.
91. Rubic T, Lametschwandtner G, Jost S, Hinteregger S, Kund J, Carballido-Perrig N, et al. Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. *Nature immunology*. 2008;9(11):1261.

92. Mincham KT, Scott NM, Lauzon-Joset J-F, Leffler J, Larcombe AN, Stumbles PA, et al. Transplacental immune modulation with a bacterial-derived agent protects against allergic airway inflammation. *Journal of Clinical Investigation*. 2018;128(11):4856-69.
93. Conrad ML, Ferstl R, Teich R, Brand S, Blümer N, Yildirim AÖ, et al. Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe *Acinetobacter lwoffii* F78. *Journal of Experimental Medicine*. 2009;206(13):2869-77.
94. Mincham KT, Jones AC, Bodinier M, Scott NM, Lauzon-Joset J-F, Stumbles PA, et al. Transplacental innate immune training via maternal microbial exposure: Role of XBP1-ERN1 axis in dendritic cell precursor programming. *Frontiers in Immunology*. 2020;11:3173.
95. Lauzon-Joset J-F, Mincham KT, Scott NM, Khandan Y, Stumbles PA, Holt PG, et al. Protection against neonatal respiratory viral infection via maternal treatment during pregnancy with the benign immune training agent OM-85. *Immunology*; 2021.
96. Macia L, Nanan R, Hosseini-Beheshti E, Grau GE. Host- and Microbiota-Derived Extracellular Vesicles, Immune Function, and Disease Development. *International Journal of Molecular Sciences*. 2019;21(1).
97. Shen Y, Giardino Torchia ML, Lawson GW, Karp CL, Ashwell JD, Mazmanian SK. Outer membrane vesicles of a human commensal mediate immune regulation and disease protection. *Cell Host & Microbe*. 2012;12(4):509-20.
98. Kim M-H, Choi SJ, Choi H-I, Choi J-P, Park H-K, Kim EK, et al. *Lactobacillus plantarum*-derived Extracellular Vesicles Protect Atopic Dermatitis Induced by *Staphylococcus aureus*-derived Extracellular Vesicles. *Allergy, Asthma & Immunology Research*. 2018;10(5):516.
99. Kim J-H, Jeun E-J, Hong C-P, Kim S-H, Jang MS, Lee E-J, et al. Extracellular vesicle-derived protein from *Bifidobacterium longum* alleviates food allergy through mast cell suppression. *Journal of Allergy and Clinical Immunology*. 2016;137(2):507-16.e8.
100. Holder B, Jones T, Sancho Shimizu V, Rice TF, Donaldson B, Bouqueau M, et al. Macrophage Exosomes Induce Placental Inflammatory Cytokines: A Novel Mode of Maternal-Placental Messaging. *Traffic*. 2016;17(2):168-78.
101. Mysorekar I, Cao B. Microbiome in Parturition and Preterm Birth. *Seminars in Reproductive Medicine*. 2014;32(01):050-5.
102. Robert-Gangneux F, Gangneux J-P, Vu N, Jaillard S, Guiguen C, Amiot L. High level of soluble HLA-G in amniotic fluid is correlated with congenital transmission of *Toxoplasma gondii*. *Clinical Immunology*. 2011;138(2):129-34.
103. Nuriel-Ohayon M, Neuman H, Koren O. Microbial Changes during Pregnancy, Birth, and Infancy. *Frontiers in Microbiology*. 2016;7.
104. Pelzer E, Gomez-Arango LF, Barrett HL, Nitert MD. Review: Maternal health and the placental microbiome. *Placenta*. 2017;54:30-7.
105. D'Argenio V. The Prenatal Microbiome: A New Player for Human Health. *High-Throughput*. 2018;7(4):38.
106. Jiménez E, Fernández L, Marín ML, Martín R, Odriozola JM, Nueno-Palop C, et al. Isolation of Commensal Bacteria from Umbilical Cord Blood of Healthy Neonates Born by Cesarean Section. *Current Microbiology*. 2005;51(4):270-4.
107. Wassenaar TM, Panigrahi P. Is a foetus developing in a sterile environment? *Letters in Applied Microbiology*. 2014;59(6):572-9.

108. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. *Scientific Reports*. 2016;6(1):23129.
109. Romero R, Miranda J, Chaemsathong P, Chaiworapongsa T, Kusanovic JP, Dong Z, et al. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2015;28(12):1394-409.
110. Romero R, Miranda J, Kusanovic JP, Chaiworapongsa T, Chaemsathong P, Martinez A, et al. Clinical chorioamnionitis at term I: microbiology of the amniotic cavity using cultivation and molecular techniques. *Journal of Perinatal Medicine*. 2015;43(1).
111. DiGiulio DB, Romero R, Amogan HP, Kusanovic JP, Bik EM, Gotsch F, et al. Microbial Prevalence, Diversity and Abundance in Amniotic Fluid During Preterm Labor: A Molecular and Culture-Based Investigation. *PLoS ONE*. 2008;3(8):e3056.
112. DiGiulio DB, Romero R, Kusanovic JP, Gómez R, Kim CJ, Seok KS, et al. Prevalence and Diversity of Microbes in the Amniotic Fluid, the Fetal Inflammatory Response, and Pregnancy Outcome in Women with Preterm Pre-Labor Rupture of Membranes. *American Journal of Reproductive Immunology*. 2010.
113. DiGiulio DB. Diversity of microbes in amniotic fluid. *Seminars in Fetal and Neonatal Medicine*. 2012;17(1):2-11.
114. Younge N, McCann JR, Ballard J, Plunkett C, Akhtar S, Araújo-Pérez F, et al. Fetal exposure to the maternal microbiota in humans and mice. *JCI Insight*. 2019;4(19):e127806.
115. Jiménez E, Delgado S, Maldonado A, Arroyo R, Albújar M, García N, et al. *Staphylococcus epidermidis*: A differential trait of the fecal microbiota of breast-fed infants. *BMC Microbiology*. 2008;8(1):143.
116. Hansen R, Scott KP, Khan S, Martin JC, Berry SH, Stevenson M, et al. First-Pass Meconium Samples from Healthy Term Vaginally-Delivered Neonates: An Analysis of the Microbiota. *PLOS ONE*. 2015;10(7):e0133320.
117. Tapiainen T, Paalanen N, Tejesvi MV, Koivusaari P, Korpela K, Pokka T, et al. Maternal influence on the fetal microbiome in a population-based study of the first-pass meconium. *Pediatric Research*. 2018;84(3):371-9.
118. Zheng J, Xiao X, Zhang Q, Mao L, Yu M, Xu J. The Placental Microbiome Varies in Association with Low Birth Weight in Full-Term Neonates. *Nutrients*. 2015;7(8):6924-37.
119. Neu J. The microbiome during pregnancy and early postnatal life. *Seminars in Fetal and Neonatal Medicine*. 2016;21(6):373-9.
120. Racicot K, Cardenas I, Wünsche V, Aldo P, Guller S, Means RE, et al. Viral Infection of the Pregnant Cervix Predisposes to Ascending Bacterial Infection. *The Journal of Immunology*. 2013;191(2):934-41.
121. Prince AL, Chu DM, Seferovic MD, Antony KM, Ma J, Aagaard KM. The Perinatal Microbiome and Pregnancy: Moving Beyond the Vaginal Microbiome. *Cold Spring Harbor Perspectives in Medicine*. 2015;5(6):a023051-a.
122. Lannon SMR, Adams Waldorf KM, Fiedler T, Kapur RP, Agnew K, Rajagopal L, et al. Parallel detection of lactobacillus and bacterial vaginosis-associated bacterial DNA in the chorioamnion and vagina of pregnant women at term. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2019;32(16):2702-10.
123. Theis KR, Romero R, Winters AD, Greenberg JM, Gomez-Lopez N, Alhousseini A, et al. Does the human placenta delivered at term have a microbiota? Results of cultivation,

- quantitative real-time PCR, 16S rRNA gene sequencing, and metagenomics. *American Journal of Obstetrics and Gynecology*. 2019;220(3):267.e1-.e39.
124. Ardisson AN, de la Cruz DM, Davis-Richardson AG, Rechcigl KT, Li N, Drew JC, et al. Meconium Microbiome Analysis Identifies Bacteria Correlated with Premature Birth. *PLoS ONE*. 2014;9(3):e90784.
 125. Petersen C, Dai DLY, Boutin RCT, Sbihi H, Sears MR, Moraes TJ, et al. A rich meconium metabolome in human infants is associated with early-life gut microbiota composition and reduced allergic sensitization. *Cell Reports Medicine*. 2021:100260.
 126. Mishra A, Lai GC, Yao LJ, Aung TT, Shental N, Rotter-Maskowitz A, et al. Microbial exposure during early human development primes fetal immune cells. *Cell*. 2021.
 127. Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, et al. Mother-to-Infant Microbial Transmission from Different Body Sites Shapes the Developing Infant Gut Microbiome. *Cell Host & Microbe*. 2018;24(1):133-45.e5.
 128. Korpela K, Costea P, Coelho LP, Kandels-Lewis S, Willemsen G, Boomsma DI, et al. Selective maternal seeding and environment shape the human gut microbiome. *Genome Research*. 2018;28(4):561-8.
 129. Mortensen MS, Rasmussen MA, Stokholm J, Brejnrod AD, Balle C, Thorsen J, et al. Modeling transfer of vaginal microbiota from mother to infant in early life. *Elife*. 2021;10.
 130. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. *Cell host & microbe*. 2015;17(5):690-703.
 131. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences*. 2010;107(26):11971-5.
 132. Roswall J, Olsson LM, Kovatcheva-Datchary P, Nilsson S, Tremaroli V, Simon M-C, et al. Developmental trajectory of the healthy human gut microbiota during the first 5 years of life. *Cell Host & Microbe*. 2021.
 133. Dogra S, Sakwinska O, Soh S-E, Ngom-Bru C, Brück WM, Berger B, et al. Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. *MBio*. 2015;6(1).
 134. Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. *Gut*. 2014;63(4):559-66.
 135. Shao Y, Forster SC, Tsaliki E, Vervier K, Strang A, Simpson N, et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature*. 2019;574(7776):117-21.
 136. Stokholm J, Thorsen J, Blaser MJ, Rasmussen MA, Hjelmso M, Shah S, et al. Delivery mode and gut microbial changes correlate with an increased risk of childhood asthma. *Sci Transl Med*. 2020;12(569).
 137. Wampach L, Heintz-Buschart A, Fritz JV, Ramiro-Garcia J, Habier J, Herold M, et al. Birth mode is associated with earliest strain-conferred gut microbiome functions and immunostimulatory potential. *Nature Communications*. 2018;9(1):5091.
 138. Korpela K, Salonen A, Vepsäläinen O, Suomalainen M, Kolmeder C, Varjosalo M, et al. Probiotic supplementation restores normal microbiota composition and function in antibiotic-treated and in caesarean-born infants. *Microbiome*. 2018;6(1):182.

139. Korpela K, Helve O, Kolho K-L, Saisto T, Skogberg K, Dikareva E, et al. Maternal Fecal Microbiota Transplantation in Cesarean-Born Infants Rapidly Restores Normal Gut Microbial Development: A Proof-of-Concept Study. *Cell*. 2020;183(2):324-34.e5.
140. Wibowo MC, Yang Z, Borry M, Hubner A, Huang KD, Tierney BT, et al. Reconstruction of ancient microbial genomes from the human gut. *Nature*. 2021.
141. Song SJ, Lauber C, Costello EK, Lozupone CA, Humphrey G, Berg-Lyons D, et al. Cohabiting family members share microbiota with one another and with their dogs. *elife*. 2013;2:e00458.
142. Strachan DP. Hay fever, hygiene, and household size. *BMJ: British Medical Journal*. 1989;299(6710):1259.
143. Zhong Y, Marungruang N, Fak F, Nyman M. Effects of two whole-grain barley varieties on caecal SCFA, gut microbiota and plasma inflammatory markers in rats consuming low- and high-fat diets. *The British journal of nutrition*. 2015;113(10):1558-70.
144. Rubic T, Lametschwandtner G, Jost S, Hinteregger S, Kund J, Carballido-Perrig N, et al. Triggering the succinate receptor GPR91 on dendritic cells enhances immunity. *Nat Immunol*. 2008;9(11):1261-9.
145. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature medicine*. 2014;20(2):159-66.
146. Szajewska H, Horvath A. Lactobacillus rhamnosus GG in the Primary Prevention of Eczema in Children: A Systematic Review and Meta-Analysis. *Nutrients*. 2018;10(9):1319.
147. Wei X, Jiang P, Liu J, Sun R, Zhu L. Association between probiotic supplementation and asthma incidence in infants: a meta-analysis of randomized controlled trials. *Journal of Asthma*. 2020;57(2):167-78.
148. Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *The Lancet*. 2001;357(9262):1076-9.
149. Kopp MV, Hennemuth I, Heinzmann A, Urbanek R. Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of Lactobacillus GG supplementation. *Pediatrics*. 2008;121(4):e850-6.
150. Wickens K, Barthow C, Mitchell EA, Stanley TV, Purdie G, Rowden J, et al. Maternal supplementation alone with *Lactobacillus rhamnosus* HN001 during pregnancy and breastfeeding does not reduce infant eczema. *Pediatric Allergy and Immunology*. 2018;29(3):296-302.
151. Boyle RJ, Ismail IH, Kivivuori S, Licciardi PV, Robins-Browne RM, Mah L-J, et al. Lactobacillus GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial: Prenatal probiotics for eczema prevention. *Allergy*. 2011;66(4):509-16.
152. Kuitunen M, Kukkonen K, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, et al. Probiotics prevent IgE-associated allergy until age 5 years in cesarean-delivered children but not in the total cohort. *Journal of Allergy and Clinical Immunology*. 2009;123(2):335-41.
153. Bertelsen RJ, Brantsæter AL, Magnus MC, Haugen M, Myhre R, Jacobsson B, et al. Probiotic milk consumption in pregnancy and infancy and subsequent childhood allergic diseases. *Journal of Allergy and Clinical Immunology*. 2014;133(1):165-71. e8.

154. Patnode ML, Guruge JL, Castillo JJ, Couture GA, Lombard V, Terrapon N, et al. Strain-level functional variation in the human gut microbiota based on bacterial binding to artificial food particles. *Cell Host Microbe*. 2021;29(4):664-73 e5.
155. Vandeputte D, Kathagen G, D'Hoe K, Vieira-Silva S, Valles-Colomer M, Sabino J, et al. Quantitative microbiome profiling links gut community variation to microbial load. *Nature*. 2017;551(7681):507-11.
156. Bodein A, Chapleur O, Droit A, Lê Cao K-A. A generic multivariate framework for the integration of microbiome longitudinal studies with other data types. *Frontiers in genetics*. 2019;10:963.
157. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome datasets are compositional: and this is not optional. *Frontiers in microbiology*. 2017;8:2224.
158. Quinn TP, Erb I, Richardson MF, Crowley TM. Understanding sequencing data as compositions: an outlook and review. *Bioinformatics*. 2018;34(16):2870-8.
159. VanderWeele TJ. Principles of confounder selection. *European journal of epidemiology*. 2019;34(3):211-9.
160. Ponsonby A-L. Reflection on modern methods: building causal evidence within high-dimensional molecular epidemiological studies of moderate size. *International Journal of Epidemiology*. 2021.
161. Shanahan F, Hill C. Language, numeracy and logic in microbiome science. *Nat Rev Gastroenterol Hepatol*. 2019;16(7):387-8.
162. Walter J, Armet AM, Finlay BB, Shanahan F. Establishing or Exaggerating Causality for the Gut Microbiome: Lessons from Human Microbiota-Associated Rodents. *Cell*. 2020;180(2):221-32.

Figure Legends

Figure 1. Putative pathways linking environmental factors, the maternal gut microbiome during pregnancy, early life immune development and protection against allergic disease and asthma. IgG: immunoglobulin G; MAMPS: microbe associated molecular patterns; Treg: regulatory T cells; Th1: T-helper 1; IRF7: interferon regulatory transcription factor 7 (IRF7); Th17: T-helper 17.

Figure 2. Hypothesized pathway by which maternal carriage of *Prevotella copri* trains fetal immunity, conveying protection against subsequent allergic disease and asthma. Treg: regulatory T cells; Th1: T-helper 1; IRF7: interferon regulatory transcription factor 7; Th1: T-helper 1.

Environment

↑ Environmental
microbial
diversity

↓ Maternal
antibiotic
exposure

↑ Maternal
dietary
fiber

↓ Maternal
psychosocial
stress

Feto-maternal

Maternal
microbiome

Placental and
meconium
microbiomes

Bacterial components

- IgG-bound
- Bacterial lysates
- MAMPs

Bacterial products

- Bacterial metabolites
- Extracellular vesicles

Increased
thymic Treg

Enhanced
dendritic cell
network

Enhanced
microbial pattern
recognition

Infancy

Enhanced
regulatory
immunity

Peripherally
induced gut and
respiratory Treg

Enhanced
Th1/IRF7/Th17

Pathogen
clearance

Infant
microbiome

Decreased allergic
disease and asthma

