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The First 1000 Days: Assembly of the Neonatal Microbiome and Its Impact on Health Outcomes

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Abstract

Early life microbial colonization is critical for the development of the immune system, postnatal growth, and long-term health and disease. The dynamic and nascent microbiomes of children are highly individualized and are characterized by low bacterial diversity. Any disruptions in microbial colonization can contribute to shifts in normal microbial colonization that persist past the first 1000 days of life and result in intestinal dysbiosis. Here, we focus on microbiome-host interactions during fetal, newborn, and infant microbiome development. We summarize the roles of bacterial communities in fetal development and adverse health outcomes due to dysbiosis. We also discuss how internal and external factors program the microbiome's metabolic machinery as it evolves into an adult-like microbiome. Finally, we discuss the limits of current studies and future directions. Studies on the early-life microbiome will be critical for a better understanding of childhood health and diseases, as well as restorative methods for the prevention and treatment of diseases in adulthood.

Keywords

Bacteria; Fungi; Immunity; Microbiome; Necrotizing enterocolitis; Probiotics; Virome

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Introduction

With the application of culture-independent techniques and high throughput technologies over the last decade and a half, the significance of the intestinal microbiome has been revealed. Alongside this discovery, has been an appreciation for the dynamic and complex colonization during the first 1000 days after conception, including pregnancy and the first 2 years of life.[1–3] Microbiome studies have defined the concepts of the microbiota (microorganisms present in a given environment) and the microbiome (genes and genomes of the microbiota, including products of the microbiota and the host environment).[4] Though initially perceived as chaotic and haphazard, research has demonstrated that early life bacterial succession is in fact a well-orchestrated series of events that when occurring correctly, can result in intestinal eubiosis.[5,6] In such cases, the intestinal microbiome can impart optimal health outcomes, not only during the first 3 years of life but also throughout later childhood and adulthood. Early-life microbial programming of the developing infant immune system is now considered a key component of the Developmental Origins of Health and Disease (DOHad), also known as the Barker hypothesis.[7,8] The DOHad hypothesis, which existed well before the advancement of human microbiome research, describes variations in health outcomes, including allergies, asthma, and hypertension, due to a cadre of environmental factors during a critical window of development. This concept now extends to the specific role that microbes may have in populating infants during early life, including their interactions with the host's developing immune system, postnatal growth, and their subsequent role in long-term health and disease.

Focusing on early life microbial colonization is critical because the early life microbiome is not stable during this window of development.[9] In contrast, the adult microbiome, is minimally impacted by external factors, including antibiotics, diet, and interval illnesses. The dynamic and nascent microbiomes of children are highly individualized and are characterized by low bacterial diversity with fewer overall bacterial species.[9] In the case of newborns and infants, any disruptions in bacterial colonization can contribute to shifts in normal microbial colonization that are not transient but persist past the first 1000 days of life. These disruptions, often result in dysbiosis, which is defined as an imbalance in the composition and metabolic capacity of microbiota and can increase the risk of chronic health conditions. Though human microbiome studies have typically focused on bacterial colonization, other organisms, including viruses (virome), fungi (mycobiome), archaea (archaeome), and bacteriophages are also dynamic during this period. All of these organisms establish themselves in the gut through interactions with bacteria and the host immune system.[10]

For the purposes of this review, we will focus on microbiome-host interactions during fetal, newborn, and infant microbiome development. We will discuss bacterial communities that may play a role in fetal development. We will describe the dynamic changes in the microbiome during the first year of life in term and preterm infants and the second year of life after the introduction of an expansive diet. We will also identify areas where we have observed adverse health outcomes due to intestinal dysbiosis early in life. Finally, we will discuss how internal and external factors program the microbiome's metabolic machinery as it evolves into a more mature, adult-like microbiome.

Factors Affecting Intrauterine Colonization and the Fetal Microbiome

Maternal Factors during Pregnancy

Many maternal factors affect the newborn microbiome, including interactions between the maternal and fetal genome that contribute to bacterial colonization and unique cross-talk between pioneer organisms and the *in utero* environment. Pregnancy is associated with a shift in the mother to a proinflammatory state which is associated with metabolic dysfunction, including insulin resistance, dyslipidemia, and hypertension.[11] It is unclear whether these changes in maternal metabolic machinery, which are beneficial to the developing fetus, are modulated by a shift in the maternal intestinal microbiome and whether subsequent effects are observed in the fetus. In some studies, despite developing a proinflammatory metabolic profile during pregnancy, the maternal microbiome remains unchanged, while in other studies an increase in the abundance of Actinobacteria and Proteobacteria, a decrease in butyrate-producing bacteria, and a decline in bacterial diversity are reported.[11,12] Further research is necessary to define how any of these changes in the maternal intestinal microbiome affect fetal development and newborn intestinal colonization (Fig. 1).

Maternal antibiotic use during pregnancy has been associated with alterations in the maternal microbiome, including increases in vaginal colonization by Staphylococcus. It is also accompanied by derangements in the newborn bacterial ecosystem, increasing the childhood risk of developing allergies, otitis media, and obesity. Another maternal factor affecting the newborn microbiome includes maternal obesity, which is correlated with a shift in the infant microbiome that can increase their risk of obesity. The intestinal dysbiosis associated with obesity in pregnant and nonpregnant adults is characterized by an imbalance in the Firmicutes-to-Bacteroidetes ratio.[13,14] The microbiome of infants born to obese mothers had a similar derangement with an increased abundance of Firmicutes, particularly from the Lachnospiraceae family. These infants also had a greater risk of becoming overweight at 1–3 years of age.[15] Mothers with gestational diabetes mellitus (GDM) have an altered gut microbiota, including *Bifidobacterium* spp., which was heritable by the fetus during pregnancy.[16,17] In one study by Wang et al., samples across multiple body sites were collected from pregnant women and their neonates. Bacterial communities in mothers with GDM and their offspring were similarly altered across body sites, consistent with an intergenerational concordance between GDM mothers and their offspring. Other associations between maternal complications, including HIV, group B Streptococcus, irritable bowel disease, and intra-amniotic infection, have been associated with inconsistent shifts in the infant intestinal microbiome. However, most studies on the fetal inheritance of the maternal microbiome demonstrate a well-orchestrated process with a lifelong impact on the newborn.

Fetal Microbiome

While the *in utero* environment was traditionally viewed as sterile, controversy now exists over whether a fetal microbiome modulated by maternal factors, including diet and immunogenetics, develops during pregnancy and impacts childhood and adult health outcomes.[18,19] With conflicting evidence on this issue, we cannot accurately identify

the initial timepoint when bacteria stimulate the development of different organ systems, including mucosal immunity. Other complexities in this debate are whether bacterial DNA that is detectable in the placenta or amniotic fluid, represents viable or dead organisms and whether its presence is sufficient to corroborate not only the presence of these microorganisms but a role in fetal development. Thus, studies have emerged on both sides of this discussion to further investigate whether bacteria influence *in utero* development.

Studies supporting colonization *in utero* include work from Aagaard et al., in which approximately 320 placental samples were collected under sterile conditions. The microbiome of these samples was compared with samples from different body sites (nares, mouth, skin, gut) in nonpregnant patients enrolled in the NIH-sponsored human microbiome project.[20] Gram-negative intracellular organisms were detected in placental samples from this study in the absence of chorioamnionitis. In addition, based on Bray Curtis similarity indices, bacterial communities in the placenta shared the greatest similarity with the oral microbiome of nonpregnant subjects.

Maternal-fetal microbiota transmission *in utero* is also supported by the detection of bacterial DNA in meconium (first stool passed by a newborn in the first few hours to days of life) from organisms that were also detected in amniotic fluid, an expected finding given how the fetus swallows amniotic fluid during gestation. A study comparing fluid and meconium demonstrated that approximately 30% of detected species were shared between amniotic fluid and meconium samples, while 30% were only found in amniotic fluid and 40% were unique to meconium. However, the 30% of shared species accounted for up to 95% of total reads and only a small percentage of reads was found in either the amniotic fluid or meconium alone.[21]

Microbiome studies of meconium shortly after delivery were also investigated in a cohort of infants undergoing bowel resections due to anatomic anomalies without evidence of infection. Unique intestinal microbial communities were identified as early as the first day of life, even in those infants delivered operatively without ever having received enteral feeds.[22]

Further studies on *in utero* colonization that define the origins of the nascent microbiome in fetuses and newborns are imperative for understanding disease processes, such as preterm labor and necrotizing enterocolitis (NEC). All of these studies will need to address concerns about contamination and incorporate negative controls into analyses to withstand the scrutiny of the scientific community. However, should fetal inheritance of the maternal microbiome prove true, it would be a unique opportunity to modulate fetal outcomes through modifications in maternal diet and other lifestyle choices during pregnancy.

Perinatal and Newborn Microbial Colonization

Delivery Mode

A large source of pioneer bacteria colonizing newborns is acquired during delivery with the mode of delivery playing a major role in the type of bacteria inherited. In one of the initial studies of ten infants, including four delivered vaginally and six delivered via cesarean section (C-section), maternal samples (skin, oral mucosa, vagina)

were collected prior to delivery along with neonatal samples (oral mucosa, nasopharynx, meconium) and environmental samples from the operating room.[23] Though bacterial communities of newborns were highly undifferentiated, vaginally delivered infants had microbiomes similar to that of maternal vaginal samples, including a higher abundance of Bacteroides. Conversely, infants born via C-sections had microbiomes similar to maternal skin and hospital organisms, including those detected in the operating room. Such studies demonstrate a shift toward aberrant gut colonization after C-sections and raise concerns about the impact of a worldwide rise in the rate of C-sections performed without maternal health indications.[24,25] Studies describing the association of operative deliveries with adverse health outcomes during childhood, including delays in cognitive development, autoimmune diseases, and atopic disorders, have generated questions about whether aberrant postnatal colonization contributes to these outcomes.[26,27] Infants born via C-section also had lower levels of *Bacteroides longum* subspecies *infantis* (*B. infantis*), a key symbiont and a common strain contained in probiotics.[28,29] *B. infantis* has co-evolved with mother-infant dyads to assist with a host of metabolic and immune functions, including complex carbohydrate digestion. In one study, infants delivered via C-sections had decreased colonization with *B. infantis* and microbial diversity. This was associated with reduced Th1 responses and neonatal immunity during the first 2 years of life.[30]

Another area related to the delivery mode that is under active debate is the question of whether the maternal vaginal vs rectal microbiome is the key driver of a newborn's postnatal colonization across all delivery modes. To investigate whether maternal or vaginal microbiome impacts colonization of the newborn after delivery one study examined 75 infants born either vaginally ($n = 40$) or by planned or emergent C-sections ($n = 35$) and found virtually undetectable levels of Lactobacillus in vaginally delivered patients, despite Lactobacillus being the most common member of the vaginal microbiome. Vaginally delivered infants were in fact seeded by more bacterial strains, however, the strains matched maternal rectal swabs.[31] Transplantation of the maternal fecal microbiota has been investigated as a potential way to reverse detrimental effects on the gut microbiota observed in infants delivered by C-sections and restore a normal gut microbiome. In a recent proof of concept study, researchers identified a cohort of seven mothers with planned C-section delivery, and maternal fecal samples were collected approximately 3 weeks prior to delivery.[32] Infants received these maternal fecal samples orally after delivery and after approximately 3 months, infants demonstrated restoration of their intestinal microbiome to mimic that of infants delivered vaginally. These studies have provided promising insight into how to reestablish normal gut colonization after an important early life event.

Differences between the Preterm and Term Infant Microbiome

Distinct differences exist between the term and the preterm intestinal microbiome, which serves as the foundation for normal immune development, postnatal growth, and subsequent health outcomes. Immaturity of the intestinal microbiome in preterm infants affects their normal metabolic capacity with impaired nutrient absorption and delayed intestinal motility. Subsequently, more than half of preterm infants are discharged with postnatal growth failure.[33] The preterm gut also has poorly developed barrier function, motility, and immune function, which makes it a source of infections and inflammation, including NEC, a

devastating bowel condition that impacts approximately 7% of preterm births. Up to 5% of all neonatal intensive care unit (NICU) admissions are affected by NEC and it is associated with significant mortality and morbidity in up to 35% of cases.[34] The gut microbiota of preterm infants is characterized by delayed bacterial colonization including decreased microbial diversity, decreased levels of commensals and obligate anaerobes, and increased detection of potentially pathogenic organisms and opportunistic bacteria, including *Enterobacter*, *Enterococcus*, and *Staphylococcus*.[35,36] Studies have demonstrated that NEC patients, compared with preterm infants without NEC, have an even more dramatic drop in microbial diversity, which may be in part due to their increased exposure to antibiotics. This significant drop in diversity allows for individual bacterial species to have a greater impact on intestinal microbial communities, which can drive the pathogenesis of NEC.[37] Other common practices that preterm infants may be introduced to which can disrupt the intestinal microbiome include C-sections, antibiotic use, and formula feedings. In the prospective fecal collection from 45 preterm infants aged birth to 60 days, gestational age was the major determinant for preterm infants' ability to achieve normal and mature gut microbiomes, including a shift towards a *Bifidobacterium* dominant microbiome. All preterm infants in this study received human milk, which is known to increase the abundance of *Bifidobacteria* in newborns. Research continues to focus on how to promote healthy gut colonization after preterm delivery, including access and utilization of breast milk (expressed or donor) and indications for probiotics.[38]

Breast Milk and Formula's Influence on the Infant Microbiome

The American Academy of Pediatrics recommends the exclusive use of human milk for the first 6 months of life.[39] This policy statement reflects the importance of early nutrition for optimal development of the immature intestinal microbiome and immune function of the gastrointestinal tract.[1] The robust composition of human milk includes but is not exclusive to antimicrobial and immunological components, prebiotic substances (lactose, human milk oligosaccharides), and live microorganisms.[40] Metabolites and microbes present in breast milk are responsible for promoting tolerance to self-antigens and the gastrointestinal tract's response to potential human pathogens. Specifically, microorganismassociated molecular pattern signaling with toll-like receptors (TLRs) promotes a tolerogenic environment with the expansion of T regulatory cells (T regs) within the intestines.[41]

In a large, multi-center study, The Environmental Determinants of Diabetes in the Young study (TEDDY), in which stool samples collected monthly from 903 children between 3 and 46 months of age were analyzed by 16S rRNA gene sequencing, infants receiving breast milk had higher levels of *Bifidobacterium* species, specifically *B. breve* and *B. bifidum*.[42] *Bifidobacterium* spp. and *Lactobacillus* spp. were present and viable in breast milk. The ability of these two organisms, transferred from breast milk, to flourish is in part due to the initial colonization of the newborn intestine by aerobic bacteria, including *Enterobacteria*, *Staphylococcus*, and *Enterococcus*. These aerobic organisms create ideal conditions for anaerobic *Lactobacillus* and *Bifidobacteria* to thrive. Breastfeeding during this window had a comparable effect on the microbiome composition, regardless of whether it was combined with formula or solids. Conversely, early cessation of breast milk was associated with faster maturation of the gut microbiome, including accelerated colonization by Firmicutes. Infants

not breastfed at all were rapidly colonized with *Escherichia coli*. While breast milk itself is a source of microbes, contact with human skin and the areola, along with the retrograde flow of the infant's oral microbiota that occurs during nursing, plays an important role as well.[43] The TEDDY study also demonstrated that *Staphylococcus* was the main organism transferred to the newborn during contact with the areola.

Environmental Factors

The importance of environmental factors, including a newborn's immediate surroundings, his family's lifestyle, and geographic location, is well-demonstrated, particularly in preterm infants residing in the NICU, who have an increased likelihood of inheriting flora from this hospital setting.[44] Inherited organisms are often those associated with nosocomial infections due to their extended exposure to the hospital environment during a critical window of development for their immune systems.[45] Outside of the hospital, an infant's environment remains an important contributor to intestinal gut colonization and has been validated in stool studies. In such studies, bacteria isolated in infant fecal samples matched those identified in their immediate environment. Family members and siblings have been shown to affect an infant's microbiome and initial colonization. For example, the number of older siblings positively correlates with bacterial diversity and richness at 18 months of age, with the increasing relative abundance of *Firmicutes* and *Bacteroidetes* in infants with more siblings.

Other differences in microbial composition between infants with siblings and those without have been observed.[46] For example, in the KOALA Birth Cohort from the Netherlands, infants with sibling exposure had a higher number of *Bifidobacteria* in their gut than those without siblings.[47] In another cohort study, infants with no siblings had an increased number of non-*E. coli enterobacteria* along with clostridia in the gut in tandem with a lower anaerobe-to-facultative anaerobe ratio.[48] More studies in this area of the "sibling effect" are warranted in order to better define the specific role of siblings during early life development.

Geographic locations are also a key consideration and differences are likely secondary to different ethno-geographic populations with distinct regional diets and cultural practices.[49] When studies of infants' gut microbiota were conducted in infants living in rural Africa, comparisons of their fecal microbiome amongst these rural babies with those living in urban Italy demonstrated the dissimilarity between the different communities.[50] Similar differences amongst the infant microbiome in other regions of the world are also apparent when exploring functional differences in bacterial communities in urban vs rural environments.[51] All of these studies demonstrate the inability to generalize microbiome studies across different family structures, countries, and home environments without taking into account such variables.

Evolution of the Microbiome during the Second Year of Life

The introduction of solid foods and cessation of breast milk or formula feeds in the second year of life is responsible for a rapid expansion in the structure and function of infants' microbiomes as their diets gradually transition to an adult-like composition.[2,52,53] This evolution in the microbiome of infants' intestinal tracts is essential for bacteria to assume

new digestive roles, including the breakdown of glycans, mucin, and complex carbohydrates, along with the production of shortchain fatty acids. In a large study of 330 Danish children whose microbiome was evaluated at 9, 18, and 36 months, as formula and breast milk were gradually discontinued, *Lactobacillaceae*, *Bifidobacteriaceae*, *Enterococcaceae*, and *Enterobacteriaceae* species abundance decreased. Simultaneously, an increase in the abundance of *Lachnospiraceae*, *Ruminococcaceae*, and *Bacteroidaceae* species occurred.[54] Such findings were concordant with another large cohort study conducted in five different countries in which similar changes were observed anywhere from 4 to 6 weeks after transitioning from milk-based feedings to a family diet.[55]

Challenges exist in discerning the changes in the nascent microbiome that are due to a shift in dietary intake with alterations in the composition and function of bacterial communities due to advancing age. With increasing age, infants have more environmental exposures (daycare and school settings, travel) and have begun to move independently with frequent oral contact-generating a constant source of microbes that they can acquire. This confounding variable was explored by the same Danish investigators in two independent cohorts of infants aged 9 months in which a comprehensive analysis of the infants' diets was conducted in parallel with 16S rRNA gene analysis of infants' fecal samples. These analyses demonstrated that microbial diversity significantly correlated with progression toward family foods. In addition, family food dietary patterns in both cohorts, including foods rich in protein and fiber, were associated with increased alpha diversity.[56] Questions remain with regard to whether a delayed introduction of solid foods and sustained breastfeeding into the first year of life compromises this growth in bacterial diversity. Conversely, it is unclear whether this expansion of bacterial species which occurs as an infant's diet diversifies is what drives the development of a healthy microbiome in early childhood.

Colonization Beyond the First 1000 Days and Its Impact on Childhood Diseases

The importance of early life colonization is secondary to the foundation it provides for optimal lifelong health. Disrupted microbial colonization in the first 2 years of life contributes to the onset of pediatric and adult diseases, including gastrointestinal pathologies, metabolic disturbances, and neurological disorders. A common feature of many of these disorders is a loss of microbial diversity and key beneficial microbes. While the predominance of *Bifidobacterium* during the first year of life in infants on a breast milk diet is a key driver of immune protection, a pivot towards a more diverse and robust microbiome in the second year of life after the introduction of complementary foods is equally as important.[3,57] The timing of this transition is exquisitely sensitive as evidenced by adverse health outcomes that result from the early loss of *Bifidobacterium*, the premature introduction of new bacterial species, or loss of microbial diversity secondary to poor-quality diets or antibiotic use.[58]

Frequent exposure to antibiotics during the first 2 years of life, even at low doses, is one of the greatest insults to the nascent microbiome. Antibiotic stewardship has grown in attempts to preserve microbial diversity during this critical window. Specifically, the use of

antibiotics during this developmental window is associated with asthma, atopic dermatitis, multiple sclerosis, inflammatory bowel disease, juvenile idiopathic arthritis, and obesity.[59,60] The "hygiene hypothesis," which describes limited exposure to microorganisms that are important for shaping the maturing immune system, has been suggested to be responsible for the increased incidence of childhood asthma and allergies. With aggressive hygiene measures that decrease the risk of early-life infections, immune tolerance is not induced, and infants have an increased risk of developing an auto-immune disorder.[61] This situation with decreased microbial exposure, decreased microbial diversity, and impaired immune tolerance, may be further exacerbated by increased hygiene measures associated with the COVID-19 pandemic.[62]

Conclusion and Future Directions

Growing recognition of how important early life intestinal colonization has improved our understanding of disease pathologies in pediatric patients as well as our ability to identify restorative methods in instances where the nascent microbiome has been disrupted, including interventions such as prebiotics, probiotics, and fecal microbial transplantation. However, many studies investigating the neonatal microbiome are limited by the homogeneity of the population sampled because recruited subjects are typically from a single-center or geographic region. Comparisons across studies are challenging due to the use of different sample types, collection methods, and omics platforms. Finally, microbiome studies of low biomass samples, such as those collected during fetal and neonatal development, are fraught with concerns for environmental and reagent contamination.[63]

Future directions should incorporate studies that explore not just interactions between the host immune system and pioneer bacteria, but also those involving other eukaryotes, including viruses and fungi. For example, while we have defined the role of mode of birth in shaping the microbiome, few studies to date have examined how birth mode affects the diversity of the early-life gut viral microbiome or "virome."[64] Viral transmission and colonization of the newborn intestinal tract, which may be modulated by similar drivers as the gut microbiome, may share an important role in facilitating bacterial colonization and promoting bacterial diversity. Conversely, maternally-derived viruses that require maternally-derived bacteria for replication (bacteriophages) might only be able to proliferate weeks to months after bacterial populations are established, as demonstrated in prospective twin studies.[65] Growth of these bacteriophage communities might occur during the first 2 years of life alongside burgeoning bacterial diversity. Over the next decade, defining these intricate interactions between viruses and bacteria may provide valuable insights into how to improve health outcomes for newborns and infants.

Another emerging area of research that may improve our understanding of this critical developmental window is the regulation of epigenetic mechanisms through crosstalk with gut microbial metabolites. Epigenetics, which is the study of phenotypic changes due to modifications in gene expression, are not directly driven by an individual's genome. Modifications do not alter the host's nucleotide sequences but involve methylation, posttranscriptional histone alterations, chromatin restructuring, and regulation of non-coding RNA.[66] While interactions between an individual's genome and environmental factors are

critical in the regulation of these epigenetic mechanisms, gut microbial metabolites may also play a role.[67,68] Further studies that elucidate interactions between an individual's microbiome and epigenome - all epigenetic marks on the DNA of a single cell - may provide the most comprehensive understanding of risk factors for adult-onset metabolic disorders.

The establishment of a biobank with multicenter participation and collection of a variety of sample types is essential for moving many of these translational research goals forward. Sites that have developed infrastructure for creating a neonatal biobank have described the feasibility of doing so through strengthening relationships between parents and clinical research teams to maintain sample stability between transfer from the clinical laboratory to the biorepository to encourage long-term participation in sample collection.[69] Large scale initiatives, including the integrative human microbiome project, which include prospective sample collection of fetal membranes, amniotic fluid, placenta, and postnatal stool, have the potential to improve our clarity of these dynamic early life events.[70]

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References

1. Nicolas A, Kenna KP, Renton AE, et al. Genome-wide analyses identify KIF5A as a novel ALS gene. Neuron 2018;97:1268–1283. DOI: 10.1016/j.neuron.2018.02.027. [PubMed: 29566793]
2. Koenig JE, Spor A, Scalfone N, et al. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A 2011;108 Suppl 1:4578–4585. DOI: 10.1073/pnas.1000081107. [PubMed: 20668239]
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;17:852. DOI: 10.1016/j.chom.2015.05.012. [PubMed: 26308884]
4. Arevalo P, VanInsberghe D, Elsherbini J, et al. A reverse ecology approach based on a biological definition of microbial populations. Cell 2019;178:820–834.e14. DOI: 10.1016/j.cell.2019.06.033. [PubMed: 31398339]
5. Bokulich NA, Chung J, Battaglia T, et al. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med 2016;8:343ra82. DOI: 10.1126/scitranslmed.aad7121.
6. Yassour M, Vatanen T, Siljander H, et al. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Sci Transl Med 2016;8:343ra81. DOI: 10.1126/scitranslmed.aad0917.
7. Osmond C, Barker DJ, Winter PD, et al. Early growth and death from cardiovascular disease in women. BMJ 1993;307:1519–1524. DOI: 10.1136/bmj.307.6918.1519. [PubMed: 8274920]
8. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet 1986;1:1077–1081. DOI: 10.1016/s0140-6736(86)91340-1. [PubMed: 2871345]
9. Yatsunenko T, Rey FE, Manary MJ, et al. Human gut microbiome viewed across age and geography. Nature 2012;486:222–227. DOI: 10.1038/nature11053. [PubMed: 22699611]
10. Vemuri R, Shankar EM, Chieppa M, et al. Beyond just bacteria: functional biomes in the gut ecosystem including virome, mycobiome, archaeome and helminths. Microorganisms 2020;8:483. DOI: 10.3390/microorganisms8040483.
11. Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell 2012;150:470–480. DOI: 10.1016/j.cell.2012.07.008. [PubMed: 22863002]

12. DiGiulio DB, Callahan BJ, McMurdie PJ, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci U S A 2015;112:11060–11065. DOI: 10.1073/pnas.1502875112. [PubMed: 26283357]
13. Kozyrskyj AL, Kalu R, Koleva PT, et al. Fetal programming of overweight through the microbiome: boys are disproportionately affected. J Dev Orig Health Dis 2016;7:25–34. DOI: 10.1017/S2040174415001269. [PubMed: 26118444]
14. Kumari M, Kozyrskyj AL. Gut microbial metabolism defines host metabolism: an emerging perspective in obesity and allergic inflammation. Obes Rev 2017;18:18–31. DOI: 10.1111/obr.12484. [PubMed: 27862824]
15. Tun HM, Bridgman SL, Chari R, et al. Roles of birth mode and infant gut microbiota in intergenerational transmission of overweight and obesity from mother to offspring. JAMA Pediatr 2018;172:368–377. DOI: 10.1001/jamapediatrics.2017.5535. [PubMed: 29459942]
16. Ponzo V, Fedele D, Goitre I, et al. Diet-gut microbiota interactions and gestational diabetes mellitus (GDM). Nutrients 2019;11:33. DOI: 10.3390/nu11020330.
17. Kuang YS, Lu JH, Li SH, et al. Connections between the human gut microbiome and gestational diabetes mellitus. Gigascience 2017;6:1–12. DOI: 10.1093/gigascience/gix058.
18. Romano-Keeler J, Weitkamp JH. Maternal influences on fetal microbial colonization and immune development. Pediatr Res 2015;77:189–195. DOI: 10.1038/pr.2014.163. [PubMed: 25310759]
19. Perez-Munoz ME, Arrieta MC, Ramer-Tait AE, et al. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: implications for research on the pioneer infant microbiome. Microbiome 2017;5:48. DOI: 10.1186/s40168-017-0268-4. [PubMed: 28454555]
20. Aagaard KM. Author response to comment on “the placenta harbors a unique microbiome”. Sci Transl Med 2014;6:254lr3. DOI: 10.1126/scitranslmed.3010007.
21. Collado MC, Rautava S, Aakko J, et al. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep 2016;6:23129. DOI: 10.1038/srep23129. [PubMed: 27001291]
22. Romano-Keeler J, Moore DJ, Wang C, et al. Early life establishment of site-specific microbial communities in the gut. Gut Microbes 2014;5:192–201. DOI: 10.4161/gmic.28442. [PubMed: 24637795]
23. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010;107:11971–11975. DOI: 10.1073/pnas.1002601107. [PubMed: 20566857]
24. Betran AP, Ye J, Moller AB, et al. Trends and projections of caesarean section rates: global and regional estimates. BMJ Glob Health 2021;6:e005671. DOI: 10.1136/bmjgh-2021-005671.
25. Betran AP, Ye J, Moller AB, et al. The increasing trend in caesarean section rates: global, regional and national estimates: 1990–2014. PLoS One 2016;11:e0148343. DOI: 10.1371/journal.pone.0148343. [PubMed: 26849801]
26. Black M, Bhattacharya S, Philip S, et al. Planned repeat cesarean section at term and adverse childhood health outcomes: a recordlinkage study. PLoS Med 2016;13:e1001973. DOI: 10.1371/journal.pmed.1001973. [PubMed: 26978456]
27. Polidano C, Zhu A, Bornstein JC. The relation between cesarean birth and child cognitive development. Sci Rep 2017;7:11483. DOI: 10.1038/s41598-017-10831-y. [PubMed: 28904336]
28. Biasucci G, Rubini M, Riboni S, et al. Mode of delivery affects the bacterial community in the newborn gut. Early Hum Dev 2010;86 Suppl 1:13–15. DOI: 10.1016/j.earlhumdev.2010.01.004.
29. Shao Y, Forster SC, Tsaliki E, et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. Nature 2019;574:117–121. DOI: 10.1038/s41586-019-1560-1. [PubMed: 31534227]
30. Jakobsson HE, Abrahamsson TR, Jenmalm MC, et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. Gut 2014;63:559–566. DOI: 10.1136/gutjnl-2012-303249. [PubMed: 23926244]
31. Mitchell CM, Mazzoni C, Hogstrom L, et al. Delivery mode affects stability of early infant gut microbiota. Cell Rep Med 2020;1:100156. DOI: 10.1016/j.xcrm.2020.100156. [PubMed: 33377127]

32. Korpela K, Helve O, Kolho KL, et al. Maternal fecal microbiota transplantation in cesarean-born infants rapidly restores normal gut microbial development: a proof-of-concept study. Cell 2020;183:324–334.e5. DOI: 10.1016/j.cell.2020.08.047. [PubMed: 33007265]
33. Grier A, Qiu X, Bandyopadhyay S, et al. Impact of prematurity and nutrition on the developing gut microbiome and preterm infant growth. Microbiome 2017;5:158. DOI: 10.1186/s40168-017-0377-0. [PubMed: 29228972]
34. Lu L, Claud EC. Intrauterine inflammation, epigenetics, and microbiome influences on preterm infant health. Curr Pathobiol Rep 2018;6:15–21. DOI: 10.1007/s40139-018-0159-9. [PubMed: 29938128]
35. Rouge C, Goldenberg O, Ferraris L, et al. Investigation of the intestinal microbiota in preterm infants using different methods. Anaerobe 2010;16:362–370. DOI: 10.1016/j.anaerobe.2010.06.002. [PubMed: 20541022]
36. Magne F, Abely M, Boyer F, et al. Low species diversity and high interindividual variability in faeces of preterm infants as revealed by sequences of 16S rRNA genes and PCR-temporal temperature gradient gel electrophoresis profiles. FEMS Microbiol Ecol 2006;57:128–138. DOI: 10.1111/j.1574-6941.2006.00097.x. [PubMed: 16819956]
37. Wang Y, Hoenig JD, Malin KJ, et al. 16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. ISME J 2009;3(8):944–954. DOI: 10.1038/ismej.2009.37. [PubMed: 19369970]
38. Nissila E, Korpela K, Lokki AI, et al. C4B gene influences intestinal microbiota through complement activation in patients with paediatric-onset inflammatory bowel disease. Clin Exp Immunol 2017;190:394–405. DOI: 10.1111/cei.13040. [PubMed: 28832994]
39. Section on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics 2012;129:e827–e841. DOI: 10.1542/peds.2011-3552. [PubMed: 22371471]
40. Andreas NJ, Kampmann B, Mehring Le-Doare K. Human breast milk: a review on its composition and bioactivity. Early Hum Dev 2015;91(11):629–635. DOI: 10.1016/j.earlhumdev.2015.08.013. [PubMed: 26375355]
41. Plunkett CH, Nagler CR. The influence of the microbiome on allergic sensitization to food. J Immunol 2017;198:581–589. DOI: 10.4049/jimmunol.1601266. [PubMed: 28069753]
42. Vatanen T, Franzosa EA, Schwager R, et al. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. Nature 2018;562:589–594. DOI: 10.1038/s41586-018-0620-2. [PubMed: 30356183]
43. Moles L, Gomez M, Heilig H, et al. Bacterial diversity in meconium of preterm neonates and evolution of their fecal microbiota during the first month of life. PLoS One 2013;8:e66986. DOI: 10.1371/journal.pone.0066986. [PubMed: 23840569]
44. Yap PSX, Chong CW, Ahmad Kamar A, et al. Neonatal intensive care unit (NICU) exposures exert a sustained influence on the progression of gut microbiota and metabolome in the first year of life. Sci Rep 2021;11:1353. DOI: 10.1038/s41598-020-80278-1. [PubMed: 33446779]
45. Brooks B, Firek BA, Miller CS, et al. Microbes in the neonatal intensive care unit resemble those found in the gut of premature infants. Microbiome 2014;2:1. DOI: 10.1186/2049-2618-2-1. [PubMed: 24468033]
46. Laursen MF, Zachariassen G, Bahl MI, et al. Having older siblings is associated with gut microbiota development during early childhood. BMC Microbiol 2015;15:154. DOI: 10.1186/s12866-015-0477-6. [PubMed: 26231752]
47. Snijders BE, Damoiseaux JG, Penders J, et al. Cytokines and soluble CD14 in breast milk in relation with atopic manifestations in mother and infant (KOALA Study). Clin Exp Allergy 2006;36:1609–1615. DOI: 10.1111/j.1365-2222.2006.02613.x. [PubMed: 17177685]
48. Adlerberth I, Strachan DP, Matricardi PM, et al. Gut microbiota and development of atopic eczema in 3 European birth cohorts. J Allergy Clin Immunol 2007;120:343–350. DOI: 10.1016/j.jaci.2007.05.018. [PubMed: 17604093]
49. Rodriguez JM, Murphy K, Stanton C, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis 2015;26:26050. DOI: 10.3402/mehd.v26.26050. [PubMed: 25651996]

50. De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci U S A 2010;107:14691–14696. DOI: 10.1073/pnas.1005963107. [PubMed: 20679230]
51. Ayeni FA, Biagi E, Rampelli S, et al. Infant and adult gut microbiome and metabolome in rural bassa and urban settlers from Nigeria. Cell Rep 2018;23:3056–3067. DOI: 10.1016/j.celrep.2018.05.018. [PubMed: 29874590]
52. Wang M, Ahrne S, Antonsson M, et al. T-RFLP combined with principal component analysis and 16S rRNA gene sequencing: an effective strategy for comparison of fecal microbiota in infants of different ages. J Microbiol Methods 2004;59:53–69. DOI: 10.1016/j.mimet.2004.06.002. [PubMed: 15325753]
53. Thompson AL, Monteagudo-Mera A, Cadenas MB, et al. Milk- and solid-feeding practices and daycare attendance are associated with differences in bacterial diversity, predominant communities, and metabolic and immune function of the infant gut microbiome. Front Cell Infect Microbiol 2015;5:3. DOI: 10.3389/fcimb.2015.00003. [PubMed: 25705611]
54. Bergstrom A, Skov TH, Bahl MI, et al. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. Appl Environ Microbiol 2014;80: 2889–2900. DOI: 10.1128/AEM.00342-14. [PubMed: 24584251]
55. Fallani M, Amarri S, Uusijarvi A, et al. Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centres. Microbiology (Reading) 2011;157:1385–1392. DOI: 10.1099/mic.0.042143-0. [PubMed: 21330436]
56. Laursen MF, Andersen LB, Michaelsen KF, et al. Infant gut microbiota development is driven by transition to family foods independent of maternal obesity. mSphere 2016;1:e00069–15. DOI: 10.1128/mSphere.00069-15.
57. Oki K, Akiyama T, Matsuda K, et al. Long-term colonization exceeding six years from early infancy of *Bifidobacterium longum* subsp. *longum* in human gut. BMC Microbiol 2018;18:209. DOI: 10.1186/s12866-018-1358-6. [PubMed: 30541439]
58. O'Neill I, Schofield Z, Hall LJ. Exploring the role of the microbiota member Bifidobacterium in modulating immune-linked diseases. Emerg Top Life Sci 2017;1:333–349. DOI: 10.1042/ETLS20170058. [PubMed: 33525778]
59. Schwartz BS, Pollak J, Bailey-Davis L, et al. Antibiotic use and childhood body mass index trajectory. Int J Obes (Lond) 2016;40:615–621. DOI: 10.1038/ijo.2015.218. [PubMed: 26486756]
60. Horton DB, Scott FI, Haynes K, et al. Antibiotic exposure and juvenile idiopathic arthritis: a case-control study. Pediatrics 2015;136:e333–e343. DOI: 10.1542/peds.2015-0036. [PubMed: 26195533]
61. Azad MB, Konya T, Maughan H, et al. Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. Allergy Asthma Clin Immunol 2013;9:15. DOI: 10.1186/1710-1492-9-15. [PubMed: 23607879]
62. Romano-Keeler J, Zhang J, Sun J. COVID-19 and the neonatal microbiome: will the pandemic cost infants their microbes? Gut Microbes 2021;13:1–7. DOI: 10.1080/19490976.2021.1912562.
63. Eisenhofer R, Minich JJ, Marotz C, et al. Contamination in low microbial biomass microbiome studies: issues and recommendations. Trends Microbiol 2019;27:105–117. DOI: 10.1016/j.tim.2018.11.003. [PubMed: 30497919]
64. McCann A, Ryan FJ, Stockdale SR, et al. Viromes of one year old infants reveal the impact of birth mode on microbiome diversity. PeerJ 2018;6:e4694. DOI: 10.7717/peerj.4694. [PubMed: 29761040]
65. Maqsood R, Rodgers R, Rodriguez C, et al. Discordant transmission of bacteria and viruses from mothers to babies at birth. Microbiome 2019;7:156. DOI: 10.1186/s40168-019-0766-7. [PubMed: 31823811]
66. Fouhy F, Ross RP, Fitzgerald GF, et al. Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. Gut Microbes 2012;3:203–220. DOI: 10.4161/gmic.20169. [PubMed: 22572829]
67. Qin Y, Wade PA. Crosstalk between the microbiome and epigenome: messages from bugs. J Biochem 2018;163:105–112. DOI: 10.1093/jb/mvx080. [PubMed: 29161429]

68. Amatullah H, Jeffrey KL. Epigenome-metabolome-microbiome axis in health and IBD. Curr Opin Microbiol 2020;56:97–108. DOI: 10.1016/j.mib.2020.08.005. [PubMed: 32920333]

69. Schleif W, Hamblin F, Everett AD, et al. Tiny bodies, big needs: prospective biobanking of neonatal clinical remnant samples. Biopreserv Biobank 2021;19:106–110. DOI: 10.1089/bio.2020.0113. [PubMed: 33481645]

70. Integrative HMPRNC. The Integrative Human Microbiome Project: dynamic analysis of microbiome-host omics profiles during periods of human health and disease. Cell Host Microbe 2014;16:276–289. DOI: 10.1016/j.chom.2014.08.014. [PubMed: 25211071]

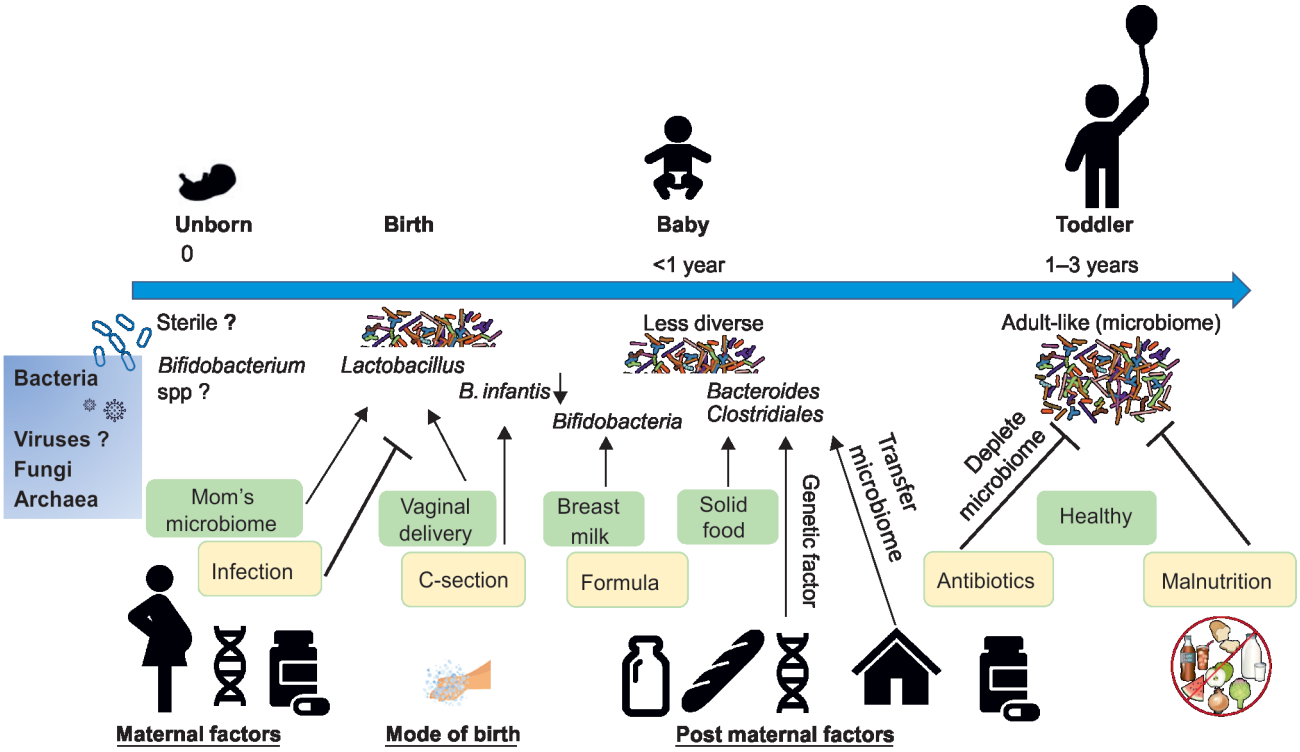

Fig. 1:

Maternal factors, modes of delivery, and postnatal factors determine the microbiome during early life. The fetus may be associated with microbes before birth. Mom's microbiome could be transported through the bloodstream to the fetus. Maternal vaginal infections could result in bacteria invading the uterine environment. The delivery method shapes the initial microbial inoculum of the newborn. Vaginally delivered infants had more *Lactobacillus and Bacteroides*. C-sections had lower levels of *Bacteroides longum* subspecies *infantis* (*B. infantis*). Postnatal factors such as antibiotic use, diet (such as human milk vs formula, and introduction of solid food), genetic factors, and environmental exposure further configure the microbiome. By age 3, the microbiome gradually shifts toward an adult-like profile. The green box indicates the positive factor to promote beneficial bacteria, and the yellow box indicates the negative factor reduces the bacterial diversity. "?" indicates controversial results or unknown. Individual bacteria associated with the different processes are indicated. The roles of viruses, fungi, and other microbes are still unknown