Microbial composition of the initial colonization of newborns

Short title: Neonatal gut colonization

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Abstract

Early life interaction with indigenous intestinal microbes is a prerequisite for healthy immune and metabolic maturation. Human infants acquire their gut microbiota predominantly from the mother. A considerable inoculum of microbes is received by the neonate during vaginal delivery. Recent observations suggest that human gut colonization may be initiated prenatally by microbes in amniotic fluid, but the significance of this phenomenon remains unknown. After birth, neonatal gut colonization is guided by human milk factors, which selectively promote the growth of specific microbes as well as by live microbes present in human milk.

Aberrant early gut colonization has been associated with increased risk of non-communicable disease in later life. Epidemiological and experimental studies suggest that the link between early gut microbiota perturbations and disease risk may be causal. Perinatal exposures including caesarean section delivery, antibiotic administration or formula feeding, which may disrupt intestinal microecology, have also been associated with development of disease. Modulation of gut microbiota in the perinatal period may offer a means to reduce the risk of chronic disease.

Introduction

At birth, the neonate enters a world inhabited by a myriad of micro-organisms. While certain bacteria, viruses and fungi represent a threat to health and well-being as potential pathogens, humans, among all other species on our planet, have evolved to live and thrive in an environment densely inhabited by microbes. During the past decades, it has become apparent that rather than mere cohabitance, our existence in the microbial world takes the form of mutually beneficial symbiosis. Indeed, every human cell with the exception of erythrocytes contains mitochondria, which are thought to originate from bacteria trapped inside our eukaryotic ancestors but now form an essential part of our energy metabolism. Furthermore, our skin and mucosal surfaces harbor a complex indigenous microbiota, which appears to be specific to both the anatomical site and the individual. The microbial community in the gastrointestinal tract and particularly the distal gut is currently most comprehensively understood. The predominant species of the gut microbiota are not frequently encountered in the environment at it is therefore apparent that we obtain our indigenous microbes from other humans and, for the most part, from the mother. The vertical microbial transmission and colonization of the infant gut is a stepwise process (**Figure 1**) disturbances of which may have deleterious consequences on health in infancy and beyond [1].

The contribution of commensal microbes for healthy immune and metabolic maturation has been the subject of rigorous scientific research over the past decade. It has become apparent that aberrant composition of the indigenous gut microbiota during early life is

associated with the risk of developing immune-mediated and inflammatory disorders including atopic disease, inflammatory bowel disease, type 1 diabetes mellitus, and obesity [reviewed in 1]. Perinatal and early life factors, which may perturb neonatal gut colonization, have also been linked to the risk of disease (**Table 1**) [1]. Consequently, elucidating the origin and optimal composition of the gut microbiota during the first days, weeks and months of life may be assumed to have significant clinical relevance.

Gut colonization at birth

The human neonate enters the extrauterine world through the birth canal, which is heavily populated with microbes. It is well-established that maternal vaginal and intestinal microbes provide an important inoculum to the neonatal gut. Vaginal lactobacilli have been shown to transiently colonize the newborn intestine only to be replaced by bacteria from other maternal sources [2,3]. The details and significance of this brief interaction with vaginal bacteria are not known. Based on studies comparing subjects born by vaginal or caesarean section (CS) delivery, it is evident that microbial contact during birth has a profound effect on gut colonization. In the immediate neonatal period, newborns born by vaginal delivery harbor a microbiota resembling that of the maternal vagina characterized by species belonging to *Lactobacillus*, *Prevotella* and *Sneathia*, whereas subjects born by CS are colonized by bacteria typically found on the skin [3]. It is evident that the maternal gut is also an important source of colonizing microbes during vaginal delivery, since 72% of the early colonizers have been reported to match the species in the maternal gut in vaginally delivered newborns as compared to 41% in

neonates born by CS delivery [4]. The gut microbiota of vaginally delivered neonates are reportedly enriched by *Bacteroides*, *Bifidobacterium*, *Parabacteroides* and *Escherichia/Shigella* species in contrast to those born by CS, in whom bacteria typically encountered in the skin and mouth as well as the environment are frequently detected [4]. Later in infancy, infants born by CS reportedly display low bacterial richness and diversity [5] and delayed colonization by Bacteroidetes [6]. Differences in gut microbiota composition between vaginally and CS delivered children have been detected until the age of seven years [7].

There are compelling epidemiological evidence to suggest that birth by CS is associated with significantly increased risk of obesity [8] and immune-mediated disease including asthma, inflammatory bowel disease and arthritis in later life [9]. It is plausible that these detrimental long-term health effects of CS are at least partially attributable to aberrant gut colonization patterns, since early gut microbiota composition has also been associated with the development of these disorders [1]. Still, it is conceivable that hormonal and immunomodulatory exposures during labor may also modulate disease risk in the offspring or that confounding factors, such as maternal obesity, increase both the likelihood of CS as mode of birth and the risk of disease in the next generation. Meticulously conducted epidemiological studies and meta-analyses are needed to address these issues. It has recently been shown that the increase in the risk of asthma is particularly pronounced following CS conducted before rupture of membranes [10], which may be interpreted to suggest a causal role for microbes in the process.

After the recognition of the significance of microbial contact during delivery for healthy immune development and the risks associated with CS, an intriguing notion of inoculating maternal vaginal microbes to the neonate has been introduced [11]. The procedure is based on the general hypothesis that seeding vaginal microbes to the newborn infant directly following birth by CS might result in early colonization resembling that of vaginally delivered neonates and, consequently, reduce the risk of long-term health problems related to CS. The vaginal seeding procedure naturally also carries the risk of transmitting pathogenic microbes such as group B *Streptococcus* or the herpes simplex virus to the neonate, and measures need to be taken to minimize these risks. Furthermore, as discussed above, the neonate is also exposed to maternal intestinal microbes during vaginal delivery and the significance of vaginal microbes for infant gut colonization remains largely unknown. Nonetheless, an interesting report from a preliminary study of 18 infants has recently been published [11]. In the 4 neonates inoculated after CS delivery by maternal vaginal microbes, the anal, oral and skin microbiota at 1 month of age appeared to resemble more closely those of vaginally delivered subjects than infants born by CS but not inoculated. Whether these changes in early colonization are associated with long-term differences in the indigenous microbiota or improved health outcomes remains to be determined by larger clinical trials.

Is the fetal gut colonized by microbes?

Several independent reports indicate that meconium, the first stool passed by the neonate but formed during fetal life, is not sterile [reviewed in 1]. Bacteria belonging

predominantly to the phylum Firmicutes as well as species representing the genera *enterobacterium*, *bifidobacterium*, *lactobacillus*, *staphylococcus*, *streptococcus* and *enterococcus* have been detected in low abundance in meconium using both traditional culture methods and culture-independent molecular techniques [12,13]. The origin of the bacteria in meconium is currently not known. It is possible that the bacteria detected in meconium are not present in the intestine *in utero* but introduced at or after birth or even after passage. Nonetheless, data from experimental animal studies indicate that the fetal mouse gut harbors viable bacteria [14]. The intrauterine origin of the microbes in meconium is also corroborated by data indicating that the duration from rupture of membranes before delivery or the time from passage to analysis does not affect meconium bacterial counts [15].

It has been suggested that the bacteria in meconium are derived from amniotic fluid swallowed during fetal life [16]. This notion was originally suggested based on comparison of microbial communities detected in meconium and previous reports on microbiota in amniotic fluid and other sites [16]. We have recently provided data demonstrating similarities between meconium and amniotic fluid microbiota from the same mother-neonate pairs [13]. The amniotic fluid samples in the study were collected during sterile elective CS delivery to minimize the possibility of contamination. Several bacterial genera including *Bacteroides, Lactobacillus, Prevotella* and *Peptostreptococcus* were detected in both amniotic fluid and meconium [13]. If the hypothesis of fetal gut colonization is further corroborated, the dogma of the sterile intrauterine compartment needs to be revised.

Small but detectable numbers of bacteria have been reported in amniotic fluid and the pregnant and non-pregnant uterus outside the context of clinical infection using both conventional culture and molecular methods [1,13]. A distinct microbiota dominated by enterobacteria has been reported in the placenta by Aagaard and colleagues [17]. Given the novelty of the hypothesis of a microbial community in the intrauterine compartment, the possibility of artifact or contamination needs to be carefully ruled out. False signal originating from contamination of the reagents used in DNA purification may be problematic particularly when analyzing samples with very low microbial abundance. A recent study using quantitative PCR failed to detect consistent results distinguishing placenta samples from controls [18]. In contrast, we have published data corroborating the presence of microbes in both placenta and amniotic fluid using both conventional culture and DGGE-PCR and sequencing of the 16S rRNA gene [13]. To date, the bacterial genera detected in amniotic fluid or placenta include among others *Propionibacterium, Enterococcus, Staphylococcus, Citrobacter* and *Lactobacillus* [13,19]. Furthermore, intriguing experimental animal studies demonstrate that labeled *Enterococcus faecium* introduced to pregnant mice may be detected in the fetal gut [14]. These data suggest bacterial transfer from the mother to the fetal intestine but our understanding of prenatal bacterial contact and its potential significance for subsequent gut colonization or later health is still virtually nonexistent.

Gut microbiota in the neonatal period and early infancy

Human milk as a modulator of gut colonization

After birth, the most significant modulator of neonatal gut colonization is breast milk. In healthy newborns, initial neonatal gut microbiota characterized by *Escherichia coli,* enterococci*,* streptococci and clostridia is rapidly followed by anaerobes including *Bifidobacterium,* and *Bacteroides* species [20-22]. It is well-established that the gut microbiota of breastfed infants is dominated by bifidobacteria [23,24], which may be detectable already during the first days of life. In contrast, formula-fed infants harbor a more diverse gut microbiota [25] resembling that of older children. Breast milk contains a large array of non-digestable oligosaccharides (human milk oligosaccharides, HMO), one of the functions of which is to selectively promote the growth of specific intestinal bacteria and particularly bifidobacteria [reviewed in 26]. *Bifidobacterium longum* subspecies *infantis* is capable of utilizing a variety of HMOs [27] and, consequently, *B. longum* is almost universally detectable in the stool of breastfed infants from various geographical locations including Northern Europe, Brazil and Malawi [28,29]. The role of HMOs in modulating gut colonization and human health is extensively reviewed elsewhere in this volume. It is of note, however, that in addition to HMOs, breast milk contains glycoproteins, which may also act as a source of selective substrates for specific bifidobacteria [30].

Human milk has been demonstrated to harbor a unique microbial community the composition of which is modulated by factors such as obesity, maternal immune-mediated disease and mode of birth [reviewed in 31]. The origin of the bacteria in human milk remains elusive, but even the non-lactating mammary gland is reportedly colonized by bacteria [31]. It is likely that many of the microbes detected in milk originate from the skin. It is of note, however, that certain microbes typically detected in human milk, including lactic acid bacteria, are also characteristic of the human gut microbiota. Intestinal origin of milk microb es is suggested by observations according to which maternal intestinal microbes may be detected in peripheral blood immune cells and breast milk during lactation [32]. Based on these data, it has been suggested that maternal gut permeability is increased during lactation and that specific intestinal microbes are transported to the mammary gland by immune cells [32]. Consistently with this notion, the probiotic *Lactobacillus reuteri* has been detected in breast milk after maternal consumption in a clinical trial [33]. The physiological function of the bacteria in breast milk is not well understood but it is possible that they function as a source of colonizers to the neonatal and infant gut. In accordance with this hypothesis, the neonatal gut microbiota shifts to bear resemblance to the microbial community in maternal milk during the first week of life [13] and there are data suggesting that breast milk microbes are transferred to the neonatal gut [34].

From an evolutionary point of view, the energy investment on HMOs on the mother's part, the profound impact of breastfeeding on early gut microbiota composition and the unique predominance of bifidobacteria in the gut microbiota during breastfeeding are

likely to provide a survival benefit for the infant. In line with this notion, epidemiological studies have linked disturbances in early gut microbiota composition and particularly reduced numbers of bifidobacteria to increased risk of developing immune-mediated or inflammatory disorders such as atopic disease and obesity [reviewed in 1] (**Table 1**). Moreover, there are data to suggest that breastfeeding exerts a protective effect against a number of chronic, non-communicable diseases [reviewed in 35]. It is possible but not proven that some of these beneficial effects of breastfeeding may result from promotion of intestinal bifidobacteria.

Antibiotic exposure

Exposure to antibiotics is known to exert a major effect on intestinal microecology and is relatively often followed by the development of diarrhea. It is alarming that antibiotic exposure in early life may also be associated with increased risk of non-communicable diseases including asthma and obesity in later life [reviewed in 1 and 36]. While it is difficult to dissect the causal relationships between antibiotic exposure, the infections against which the antibiotics have been administered and the development of chronic disease, it has been suggested based on both epidemiological and sophisticated experimental data, that aberrant gut microbiota composition resulting from early antibiotic exposure plays a causal role in the pathogenesis of obesity [reviewed in 36]. Given the significance of the neonatal period in gut colonization discussed above, it is of note that antibiotics are administered to 33-39% of mothers during delivery to prevent bacterial infection in the mother and the neonate [37,38]. To date, relatively little is

known about the impact of perinatal antibiotic exposure to gut colonization. A study based on 84 mother-infant pairs suggests that antibiotic prophylaxis administered because of maternal colonization with group B *Streptococcus* is associated with lower numbers of bifidobacteria as detected by quantitative PCR in neonatal stool samples at the age of seven days [39] but no differences were observed at the age of 30 days.

Early-onset neonatal sepsis is a devastating disease initially presenting with non-specific symptoms or signs often followed by rapid deterioration. According to current guidelines, all infants with signs or symptoms suggesting sepsis as well as certain asymptomatic individuals with a high risk of infection based on presence of factors such as chorioamnionitis should be subjected to empirical antibiotic therapy [36]. A large proportion of neonates (approximately 5%) therefore receive broad-spectrum antibiotics during the first days of life [38].

Neonatal exposure to antibiotics has been reported to result in an increase in fecal Proteobacteria and particularly Enterobacteriacaea during the first weeks of life [40-42]. In addition, a significant decrease in microbial diversity and in the abundance of *Bifidobacterium* have both been reported in neonates subjected to antibiotic intervention [40]. Our unpublished data suggest that the gut microbiota perturbations caused by neonatal antibiotic exposure persist at least until the age of 6 months but whether perinatal antibiotic exposure has an impact on risk of disease in later life is currently not known.

Prematurity

Preterm newborns subjected to care in neonatal intensive care units exhibit aberrant gut microbiota composition in early life. Low diversity of the gut microbiota and delayed colonization with bifidobacteria have been associated with being born preterm [43,44]. These disturbances may result directly from intestinal and immunologic immaturity. On the other hand, detrimental exposures including CS delivery, formula feeding and early antibiotic exposure, tend to cluster in preterm infants. Our unpublished observations suggest that while mode of birth, antibiotic exposure and formula feeding all have an impact on fecal bifidobacteria in late preterm infants, prematurity *per se* independently modulates *Bifidobacterium* colonization. Aberrant gut colonization in preterm infants has been suggested to be causally related to the risk of developing necrotizing enterocolitis [45] (**Table 1**) but whether the increase in metabolic risk factors associated with preterm birth is mediated by perturbations of early gut colonization remains unknown.

Conclusions

The composition of the early human gut microbiota has been associated with the development of non-communicable disease in later life [reviewed in 1]. Both reduced diversity and altered abundance of specific members of the intestinal ecosystem have been linked with various disorders ranging from atopic disease and obesity to infantile colic and necrotizing enterocolitis (**Table 1**). In addition, perinatal factors including CS delivery, antibiotic exposure and formula feeding have been linked to both aberrant gut

colonization patterns and increased risk of chronic disease later in childhood (**Figure 1**, **Table 1**). Data from clinical and experimental studies may be interpreted to suggest that the link between early gut microbiota composition and disease risk may at least in part be causal but our understanding of the interactions between the indigenous microbial community and ourselves, the host, are by no means complete. It is to be hoped that rigorous basic, translational and clinical research will unravel these complex phenomena.

The potential causal role of early gut microbiota composition in the development of disease underscores the importance of supporting healthy gut colonization by reducing caesarean section rates, prudent use of antibiotics and promotion of breastfeeding. In addition, the intriguing possibility of reducing disease risk by modulating early microbial contact by prebiotics or probiotics deserves to be assessed. Thus far the most convincing scientific evidence has been obtained from studies assessing the efficacy of early probiotic intervention in reducing the risk of atopic dermatitis [46] and necrotizing enterocolitis [47] in high-risk infants. Future studies will show whether prebiotics or probiotics are effective in the prevention of other chronic conditions including obesity.

References

1. Rautava S, Luoto R, Salminen S, Isolauri E: Microbial contact during pregnancy, intestinal colonization and human disease. Nat Rev Gastroenterol Hepatol 2012;9:565-576.
2. Matsumiya Y, Kato N, Watanabe K, Kato H: Molecular epidemiological study of vertical transmission of vaginal Lactobacillus species from mothers to newborn infants in Japanese, by arbitrarily primed polymerase chain reaction. J Infect Chemother 2002;8:43-49.
3. 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 USA 2010;107:11971-11975.
4. Bäckhed F, Roswall J, Peng Y, et al: Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 2015;17:690-703.
5. Azad MB, Konya T, Persaud RR, et al: Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG 2016;123:983-993.
6. 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.
7. Salminen S, Gibson GR, McCartney AL, Isolauri E: Influence of mode of delivery on gut microbiota composition in seven year old children. Gut 2004;53:1388-1389.

8. Kuhle S, Tong OS, Woolcott CG: Association between caesarean section and childhood obesity: a systematic review and meta-analysis. Obes Rev 2015;16:295-303.
9. Sevelsted A, Stokholm J, Bønnelykke K, Bisgaard H: Cesarean section and chronic immune disorders. Pediatrics 2015;135:e92-98.
10. Sevelsted A, Stokholm J, Bisgaard H: Risk of Asthma from Cesarean Delivery Depends on Membrane Rupture. J Pediatr 2016;171:38-42.
11. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, et al: Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nat Med 2016;22:250-253.
12. Moles L, Gómez 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.
13. 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. Sci Rep 2016;6:23129.
14. Jiménez E, Marín ML, Martín R, et al: Is meconium from healthy newborns actually sterile? Res Microbiol 2008;159:187-193.
15. Hansen R, Scott KP, Khan S, et al: First-Pass Meconium Samples from Healthy Term Vaginally-Delivered Neonates: An Analysis of the Microbiota. PLoS One 2015;10:e0133320.
16. Ardissone AN, de la Cruz DM, Davis-Richardson AG, et al: Meconium microbiome analysis identifies bacteria correlated with premature birth. PLoS One 2014;9:e90784.

17. Aagaard K, Ma J, Antony KM, et al: The placenta harbors a unique microbiome. Sci Transl Med 2014;6:237ra65.
18. Lauder AP, Roche AM, Sherrill-Mix S, et al: Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. Microbiome 2016;4:29.
19. 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;7:6924-6937.
20. Favier CF, de Vos WM, Akkermans AD: Development of bacterial and bifidobacterial communities in feces of newborn babies. Anaerobe 2003;9:219-229.
21. Solís G, de Los Reyes-Gavilan CG, Fernández N, Margolles A, Gueimonde M: Establishment and development of lactic acid bacteria and bifidobacteria microbiota in breast-milk and the infant gut. Anaerobe 2010;16:307-310.
22. Jost T, Lacroix C, Braegger CP, Chassard C: New insights in gut microbiota establishment in healthy breast fed neonates. PLoS One 2012;7:e44595.
23. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, et al: Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 2000;30:61-67.
24. Roger LC, Costabile A, Holland DT, Hoyles L, McCartney AL: Examination of faecal Bifidobacterium populations in breast- and formula-fed infants during the first 18 months of life. Microbiology 2010;156:3329-3341.

25. Azad MB, Konya T, Persaud RR, et al: Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG 2016;123:983-993.
26. Bode L: Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology 2012;22:1147-1162.
27. Underwood MA, German JB, Lebrilla CB, Mills DA: Bifidobacterium longum subspecies infantis: champion colonizer of the infant gut. Pediatr Res 2015;77:229-235.
28. Grześkowiak Ł, Collado MC, Mangani C, et al: Distinct gut microbiota in southeastern African and northern European infants. J Pediatr Gastroenterol Nutr 2012;54:812-816.
29. Grześkowiak Ł, Sales Teixeira TF, Bigonha SM, Lobo G, Salminen S, Ferreira CL: Gut Bifidobacterium microbiota in one-month-old Brazilian newborns. Anaerobe 2015;35:54-58.
30. Karav S, Le Parc A, Leite Nobrega de Moura Bell JM, et al: Oligosaccharides Released from Milk Glycoproteins Are Selective Growth Substrates for Infant-Associated Bifidobacteria. Appl Environ Microbiol 2016;82:3622-3630.
31. Collado MC, Rautava S, Isolauri E, Salminen S: Gut microbiota- source of novel tools to reduce the risk of human disease? Pediatric Research 2015;77:182-188.
32. Perez PF, Doré J, Leclerc M, et al: Bacterial imprinting of the neonatal immune system: lessons from maternal cells? Pediatrics 2007;119:e724-732.

33. Abrahamsson TR, Sinkiewicz G, Jakobsson T, Fredrikson M, Björkstén B: Probiotic lactobacilli in breast milk and infant stool in relation to oral intake during the first year of life. J Pediatr Gastroenterol Nutr 2009;49:349-354.

34. Jost T, Lacroix C, Braegger CP, Rochat F, Chassard C: Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. Environ Microbiol 2014;16:2891-2904.

35. Rautava S, Walker WA: Academy of Breastfeeding Medicine founder's lecture 2008: breastfeeding - an extrauterine link between mother and child. Breastfeed Med 2009;4:3-10.

36. Turta O, Rautava S: Antibiotics, obesity and the link to microbes - what are we doing to our children? BMC Med 2016;14:57.

37. Stokholm J, Schjørring S, Pedersen L, et al: Prevalence and predictors of antibiotic administration during pregnancy and birth. PLoS One 2013;8:e82932.

38. Persaud RR, Azad MB, Chari RS, Sears MR, Becker AB, Kozyrskyj AL: Perinatal antibiotic exposure of neonates in Canada and associated risk factors: a population-based study. J Matern Fetal Neonatal Med 2015;28:1190-1195.

39. Corvaglia L, Tonti G, Martini S, et al: Influence of Intrapartum Antibiotic Prophylaxis for Group B Streptococcus on Gut Microbiota in the First Month of Life. J Pediatr Gastroenterol Nutr 2016;62:304-308.

40. Fouhy F, Guinane CM, Hussey S, et al: High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin. Antimicrob Agents Chemother 2012;56:5811-5820.

41. Arboleya S, Sánchez B, Milani C, et al: Intestinal Microbiota Development in Preterm Neonates and Effect of Perinatal Antibiotics. J Pediatr 2015;166:538-544.
42. Greenwood C, Morrow AL, Lagomarcino AJ, et al: Early empiric antibiotic use in preterm infants is associated with lower bacterial diversity and higher relative abundance of Enterobacter. J Pediatr 2014;165:23-29.
43. Magne F, Abély M, Boyer F, Morville P, Pochart P, Suau A: 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.
44. Arboleya S, Binetti A, Salazar N, et al: Establishment and development of intestinal microbiota in preterm neonates. FEMS Microbiol Ecol 2012;79:763-772.
45. Neu J, Walker W: Necrotizing enterocolitis. N Engl J Med 2011;364:255-264.
46. Isolauri E, Rautava S, Salminen S: Probiotics in the development and treatment of allergic disease. Gastroenterology Clinics of North America 2012;41:747-762.
47. AlFaleh K, Anabrees J: Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 2014;4:CD005496.

Figure 1. The development of early gut microbiota.

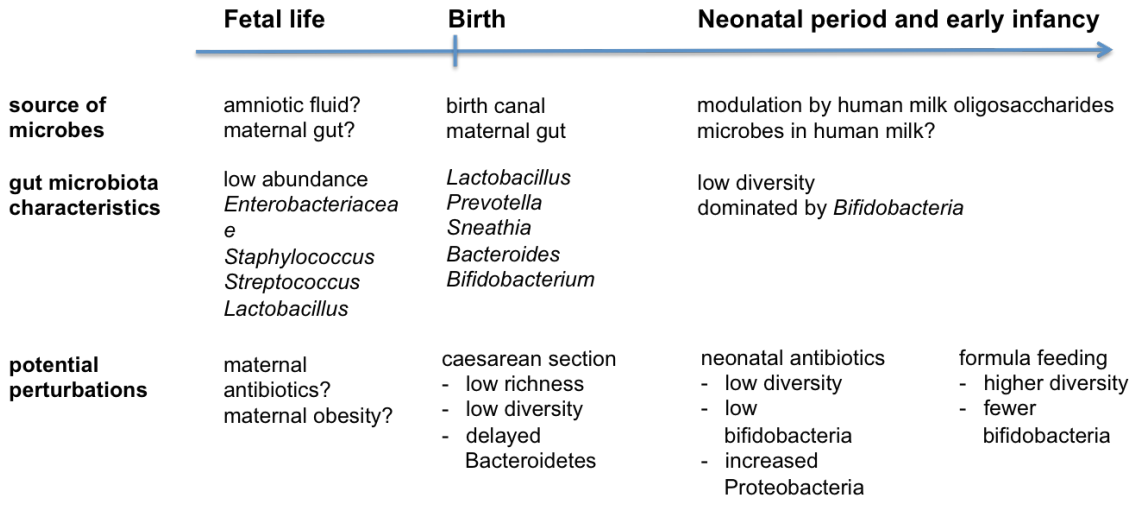

Table 1. The association between early gut microbiota composition and the risk of disease (AB=antibiotic administration, CS=caesarean section delivery).

	characteristics of early gut microbiota preceeding disease	*evidence of causality*
atopic disease	↓ diversity ↓ bifidobacteria ↓ enterococci ↑ *Escherichia coli* ↑ *Clostridium difficile*	experimental animal models ↑ after early AB ↑ after CS ↓ by probiotics
obesity and overweight	↓ bifidobacteria ↑ *Bacteroides fragilis*	experimental animal models ↑ after AB ↑ after CS
necrotizing enterocolitis	↓ Firmicutes ↑ Proteobacteria ↑ clostridia	experimental animal models ↑ after early AB ↓ by breast milk ↓ by probiotics
infantile colic	↓ diversity ↓ bifidobacteria ↓ lactobacilli ↑ Proteobacteria	↓ by probiotics