Gut Microbiota Composition in Healthy Japanese Infants and Young Adults Born by C-Section

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Keywords

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Abstract

Our gut microbiome plays a fundamental role in our health and disease. The microbial colonization of human gut begins immediately at birth and is an indispensable natural process that modulates our physiology and immunity. Recent studies are elegantly revealing how and when these microbes colonize the gut and what elements could potentially influence this natural phenomenon. The vertical mother-to-baby transmission of microbes is a crucial factor for normal development and maturation of newborn's immune, metabolic as well as neurological health. This important and delicate process of gut microbiota development may be impacted by various factors such as birth mode, type of feeding, gestational age at birth, antibiotics exposure in early life, surrounding environment and hygiene settings, and so on. Perturbations in early life gut microbial colonization have been associated with the development of several diseases such as diabetes, obesity, asthma, allergies, celiac disease, neurode-

velopmental disorders, and so on. However, it remains unclear whether predisposition to these diseases is due to the lack of acquisition of the mother's (vaginal and perianal) microbes during birth or because of abnormal exposure to unsolicited bacteria. Hence, studies are required to scrutinize the colonization pattern of infant gut microbiome in context to birth mode and also to elucidate how long these differences could persist. In these contexts, we review and discuss some of the findings obtained from recent investigation of the gut microbiota composition in healthy Japanese infants and young adults born vaginally or by C-section.

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Introduction

Recent studies have evidenced that the trillions of microorganisms that inhabit our gut influence our present as well as future health and could also account for the mounting incidences of various diseases at present troubling global population including children, adults, and elderly. It has become clear that cesarean delivery and inadequate breastfeeding can instigate abnormal spectrum of gut mi-

crobiome composition in the newborn's gut and may also account for the rising incidences of several serious health problems in children, including asthma, allergies, celiac disease, diabetes, obesity, and so on [1–6]. These maladies, among others, are more likely to occur in cases wherein an infant's gut acquires inadequate population of health-promoting bacteria and instead is predominated by unsolicited bacteria. Accordingly, like always, the obstetrics, pediatrics, and parenting advisors have again seriously started advocating fewer scheduled cesarean births and more pronounced breastfeeding exclusively for the first 6 months of life to naturally enrich the types and levels of bacteria that inhabit the newborn's gut. These gut microbes perform fundamental functions including digestion of complex nutrients, vitamins production, stimulation of immune development, checking of harmful bacteria, and nurturing normal maturation of the gut. Serious perturbation in one or more of these early life gut microbial functions could lead to severe health problems of the host.

Recent studies have evidenced that the infant gut is inhabited by several microbes in-utero [3, 4, 7, 8], although the bacteria acquired during birth and the first few months of life have the paramount impact on those that become permanent inhabitants in the gut. Recent studies have also clearly shown that both a vaginal delivery and exclusive breastfeeding can strongly influence the spectrum of gut microbiota population and the possibility of developing many health problems including asthma, inflammatory bowel disease, obesity, type 2 diabetes, systemic connective tissue disorders, juvenile arthritis, immune deficiencies, leukemia, and so on. Babies born vaginally predominantly acquire bacteria from maternal vaginal and perianal flora. However, those delivered via C-section acquire a heavy inoculum of bacteria from the maternal skin and the surrounding hospital environment. Such differences in the gut microbiota as a result of unnatural delivery have been found to persist in children until several years of life.

The rate of cesarean-section delivery has been increasing rapidly worldwide as a result of modernization of life-style. The frequency of C-section in the United States has escalated to 48% since 1996, reaching a level of about 32% in 2007 [9]. In China, the frequency of C-section delivery has increased from 5% in the 1970s to more than 60% in some hospitals [10]. Brazil is also following the same trend, with some hospitals approaching about 80% rate of cesarean-delivery [11]. Although mostly pronounced in developed and western countries, this trend is now being observed in almost all the countries worldwide, including the developing nations. While most cases of C-section are un-

avoidable and are performed for clinical indications, many cases are simply due to maternal request and might impose health risks for both mother as well as the newborn.

Gut Microbiota Composition in Healthy Japanese Infants and Young Adults: Insights from a Highly Sensitive and Quantitative Analytical Approach

Despite widespread investigations of the early life microbiome worldwide, the precise succession of many gut bacterial groups during infancy and early childhood remains to be demonstrated, particularly in terms of their actual population levels in the gut, that is, the absolute bacterial count. Also, a majority of the studies perform microbiome analysis either at single time-point during early life or in relatively small birth cohorts and hence may not provide precise data because the gut microbiome composition – though relatively simple – remains unstable and fluctuating during early life. Furthermore, most of the longitudinal studies employ bacterial rRNA gene-based polymerase chain reaction (PCR) or sequencing approaches. Despite being elegantly elaborative and comprehensive, these approaches are largely qualitative in nature and may not yield numerical data of actual bacterial counts. Also, sequence resolution may not be sufficient for the analyses of several subdominant but important species such as *Clostridium perfringens* or species belonging to the genera *Lactobacillus*, *Bifidobacterium*, and so on. Moreover, the data from DNA-based methods might also take in background DNA (e.g., DNA of dead bacteria, free bacterial DNA) present in the fecal specimen and hence may not really discriminate between viable versus dead bacteria. Consequently, the quantitative data about the early-life intestinal microbiota composition remains relatively limited and disparate. Furthermore, postnatal elements such as birth and feeding modes might not have a global influence on the overall microbiome spectrum but may still specifically impact the carriage of specific subdominant bacterial inhabitants, thereby underlining the significance of quantifying the gut microbiota in larger cohorts. In this milieu, our research group has developed and established a highly sensitive quantitative analytical system (YIF-SCAN; Yakult Intestinal Flora-SCAN) that utilizes reverse transcription-quantitative PCR to quantify the intestinal microbiota [12, 13]. The system targets the ribosomal RNA “molecules,” (which are present in abundant copies in the bacteria) and hence is able to provide a detection limit that is at least hundreds of times more sensitive (detection limits: 10^{2-3} cells/g feces) than conventional

DNA-based methods, thereby enabling highly sensitive, specific and accurate detection and quantification of viable bacteria in various types of samples such as meconium, feces, vaginal swabs, blood, and so on [12–16].

Using this unique and potential analytical system, we recently investigated and demonstrated a quantitative perspective of the age-related dynamics of the progression of gut microbiota in healthy full-term Japanese infants ($n = 89$) prospectively from birth to 3 years of age, in particular context to the birth mode. In addition, we also utilized this analytical system to generate a quantitative profile of the gut microbiota composition in healthy Japanese young adults ($n = 149$; age: 18–22 years). In this study, we briefly review and discuss how the gut microbiota composition in these cohorts differs in a particular context to the mode of birth, that is, vaginal delivery versus cesarean section.

Early-Life Gut Microbiota in Relation to the Mode of Delivery

For decades, babies were believed to be born sterile, but recent studies suggest that the neonate is already exposed to various bacteria in utero. However, the quantitative population levels of such gut bacteria, particularly with regard to the birth mode, are relatively sparse. In our quantitative investigation of the gut microbiota composition in Japanese infants, we detected several bacterial clades, albeit with varying detection rates and numbers, in the first-pass meconium of 95% infants [17], which suggests that several bacterial communities inhabit the intrauterine environment and may provide the pioneer inoculum for gut microbiota colonization. Notably, previous studies on full-term babies reported the detection of bacteria in about 65–75% of total babies studied [18, 19]. This difference may be ascribed to differences in the detection sensitivity of the methods used and hence support the advantage of the high sensitivity of our real-time quantitative (RT-qPCR)-based approach. We found *Bacteroides fragilis* group, *Enterobacteriaceae*, *Enterococcus*, *Streptococcus*, and *Staphylococcus* to be the most prevalent dwellers, with counts ranging from 10^{5-8} cells/g sample, followed by *bifidobacteria*, *lactobacilli*, *Clostridium coccoides* group, *Clostridium. leptum*

subgroup, *C. perfringens*, *Atopobium* cluster, and *Prevotella* (Fig. 1). These data demonstrated that the facultative anaerobes flourish – and hence are the first colonizers – in the nascent gut, thereby indicating a relatively aerobic intestinal environment. This also indicated that the primitive microbiota core is predominated by facultative anaerobes including *Proteobacteria*, *Staphylococci*, *Streptococci*, *Enterococci*, and so on, whereas obligate anaerobes such as Firmicutes, Bacteroidetes, and Actinobacteria start prevailing during infancy and early childhood (Fig. 1) [17].

Interestingly, we found that compared to the meconium of vaginally born babies, the meconium of cesarean-born babies is significantly less often colonized with *Lactobacillus* genus [17]. Further inspection of *Lactobacillus* species revealed that *L. gasseri* subgroup is the most prevalent subgroup in the meconium of these babies; but again, the carriage of *L. gasseri* subgroup is significantly lower in the meconium of cesarean-born babies compared to those born vaginally. We found this difference to persist up to 6 months of age but not at 3 years of age. Interestingly, by using the same RT-qPCR assays, our colleagues reported that the vaginal microbiome of young Japanese women is predominated by *Lactobacillus* groups among which the *L. gasseri* subgroup is a major component [15]. In addition, the presence of lactobacilli is rarely noticed in prenatal niches including amniotic fluid and placenta. Together, these facts clearly indicate a profound mother-baby vertical transmission of vaginal Lactobacilli during vaginal delivery, while indicative of a delayed seeding of lactobacilli in babies born by C-section. We also noticed that the overall ratio of facultative versus obligate anaerobes was significantly lower in cesarean-born babies than that in those born naturally (Fig. 1). Again, assuming vaginally born infants as healthy controls, this might indicate an imbalanced microbiota (dysbiosis) in cesarean-born babies and might hint that the course of cesarean-associated gut dysbiosis might already instigate as early as the first day of life. While the origin and sources of these bacteria in the meconium remain largely indistinct, they might be pondered to be of intra-uterine origin and suggestive of amniotic fluid. Provided that the meco-

Fig. 1. Summary of reverse-transcription-qPCR-based quantitative assessment of various major bacterial groups (*Clostridium coccoides* group, *C. leptum* subgroup, *Bacteroides fragilis* group, *Bifidobacterium*, *Atopobium* cluster, *Prevotella*, *Clostridium perfringens*, *Lactobacillus*, *Enterococcus*, *Enterobacteriaceae*, *Enterococcus* and *Staphylococcus*) in healthy Japanese infants ($n = 89$) at different time points during the first 3 years of life. Bacterial count is expressed as the mean of $\log_{10}$ cells/g feces. Prevalence (detection

rate, %) is expressed as the percentage of infants in which the specific bacterium was detected. Relative proportions of different bacteria are expressed as the percent of the total fecal bacterial count enumerated by reverse-transcription-qPCR based quantitation of the 16S rRNA gene. Age (x-axis): 1 day, 7 days, 1 month, 3 months, 6 months, 3 years. Detailed methods and results on this assessment and cohort can be found in Nagpal et al. [17, 20, 21] and Tsuji et al. [30].

(For figure 1 see next page.)

nium microbiome may indicate several characteristics of maternal health and might also be related with several infant illnesses, further research on the features of these pioneer bacteria and their crosstalk with the host would lead to novel interventions and remedies for neonate's health.

The follow-up of other bacterial groups in this cohort revealed that the intestinal carriage of *B. fragilis* group, *bifidobacteria* and several other bacteria remain inconsistently lower in cesarean-born babies until 6 months of age, but all these differences appear at or after 1 week of life [20]. Collectively, these data indicate that while the infant microbiota is certainly influenced by birth mode, these differences (except lactobacilli) do not manifest at meconium stage and become noticeable afterwards. This is intriguing and underscores the need of further research, for the reason that if the earliest population and spectrum of bacteria in vaginally born and cesarean babies is similar, then what is it that instigates and stimulates the salient alterations in the intestinal carriage of specific bacteria (such as *B. fragilis* group, *bifidobacteria*, etc.) during the following stages of life?

We noticed that the infants born via C-section have significantly higher carriage of alpha-toxigenic *C. perfringens*, as compared to vaginally born counterparts [20]. Interestingly, we also noted a strong negative correlation of *C. perfringens* with *B. fragilis* group, *lactobacilli*, *bifidobacteria*, *Atopobium* cluster, and *Prevotella*; that is, the babies that have a high count of these groups have a very low carriage of toxigenic *C. perfringens* and vice versa [20]. In addition, we also noted that babies born by cesarean-section have a significantly lower level of short-chain fatty acids (SCFAs) in their feces and hence have significantly higher fecal pH as compared to vaginally born counterparts. This clearly shows that vaginal delivery promotes higher (or normal) population of gut commensals, that is, *B. fragilis*, *lactobacilli*, and *bifidobacteria* that yield SCFAs and maintain a lower intestinal pH that eventually checks the growth of opportunistic pathogens or indigenous pathobionts such as toxigenic *C. perfringens*, whereas cesarean-born babies are affected by the lower seeding of these commensals and hence have lower intestinal SCFAs levels and a higher intestinal pH, which directly or indirectly provides *C. perfringens* an opportunity to flourish in the gut (Fig. 2).

In another study focused specifically on the quantification of 8 signature infant-associated *Bifidobacterium* species (*B. longum*, *B. breve*, *B. bifidum*, *B. catenulatum* group, *B. infantis*, *B. adolescentis*, *B. angulatum*, and *B. dentium*), we reported that compared to infants born vag-

inally, cesarean-born infants have a noticeably lower carriage of major bifidobacterial groups during the first 3 months [21]. In addition, cesarean-born infants exhibit lower/delayed carriage of several bifidobacterial members, primarily *B. catenulatum* group, *B. dentium*, and *B. infantis*, during the first 6 months of life, and the number of *Bifidobacterium* species detected during the first 6 months also remains lower in cesarean-born infants [21]. However, again, these differences tend to disappear by 3 years of age. Interestingly, we were able to detect at least one or more *Bifidobacterium* species in the meconium specimen of 21% babies. Like *lactobacilli*, *bifidobacteria* are also generally supposed to transmit vertically from mother to newborn during vaginal delivery. However, we find a comparable carriage of bifidobacteria in the meconium of vaginally or cesarean-born babies, suggesting that some bifidobacterial groups colonize the infant gut before birth. This suggests that the mother-baby transmission of bifidobacteria does occur to some extent even in cesarean-born babies, but the incidence and/or array of this transmission is relatively lower compared to that in the case of vaginal birth. Yet, these data hint that most of the bifidobacterial species in the newborn gut are acquired from the mother and that the birth mode could influence the evolution of these species during first few months of life [21].

Gut Microbiota Composition in Healthy Young Adults Born Vaginally or by C-Section

Although we found that the differences in early-life gut microbiota tend to gradually decrease and eventually vanish by 3 years of age, some studies have reported that the cesarean-associated bacterial indications may last even until 7 years of age [22], suggesting that the early-life colonization might deliberate a competitive advantage to the microbial communities associated with the birth mode. Since such differences in the early-life microbiota might influence whole-of-life health and disease predisposition, the clinical significance of these differences needs to be explicated fully; however, it remains ambiguous how long-lasting these differences in the microbiota could be. In this context, we had this unique opportunity to quantitatively assess the intestinal microbiota composition in healthy young adults ($n = 149$) and explore whether the gut microbiota differs between young adults born by C-section versus vaginal delivery. We did not notice any significant difference in the body-weight, body mass index, height, and male-female ratio by the mode of birth. However, interestingly (albeit not surprisingly), we found that the subjects in the cesarean group have sig-

Fig. 2. Schematic summary suggesting how cesarean-associated gut dysbiosis in terms of the population levels of gut commensal and beneficial clades including *B. fragilis* group, *bifidobacteria*, and *lactobacilli* can lead to higher carriage of *C. perfringens* in the infant

gut as observed during quantitative assessment of the major bacterial groups in healthy Japanese infants ($n = 89$) from day 1 to 3 years of life. Detailed methods and results on this assessment and cohort can be found in Nagpal et al. [20].

nificantly lower carriage rate of *B. fragilis* group and *L. sakei* subgroup as compared with the vaginal-delivery group (unpublished data). This might suggest that some of the differences in early-life microbiota as a result of delivery mode might persist even into young adulthood, even though the differences were observed in the carriage rate – but not population levels – of only 2 components, that is, *B. fragilis* group and *L. sakei* subgroup, both of which were found to be significantly lower in cesarean-born subjects. Of particular interest and something that is intriguing is the difference in the carriage rate of *B. fragilis* group. While innumerable studies have reported differences in the infant microbiota as a result of cesarean-delivery and there have been certain inconsistencies among these reports, the one finding that has always been unanimously and universally consistent, coherent, and reproducible is the lower/delayed colonization of *Bacteroides* in cesarean-born babies. In this context, our finding that the difference in the prevalence of *B. fragilis* group due to cesarean-delivery might persist even until young adult age is intriguing and warrants further investigation of *Bacteroides* carriage and also on the fluctuation and stabilization of the gut microbiota, particularly because *B. fragilis* is known to exert strong effects on the host immune system [23, 24]. We also noticed relatively

lower levels of propionate in the feces of subjects in the cesarean-group (unpublished data), which again is interesting and might be linked to the corresponding lower prevalence of *B. fragilis* because *Bacteroides* are known to yield propionate by utilizing succinate a substrate [25]. Interestingly, both bacteroides and propionate have been implicated, in separate studies, in diet-induced obesity by affecting gut hormones and food intake [25, 26]. More interestingly, infants born by C-section are often found to be at more risk for various diseases such as asthma, obesity, diabetes, and so on [27–29]. In our study on Japanese infants, we also found that the levels of propionate in infants delivered via cesarean-section remain lower intermittently during the first 6 months of life, as compared to vaginally delivered babies [30]. In these contexts, our finding of lower carriage of both *B. fragilis* group and propionate in the cesarean-born group of young adults is really intriguing and deserves further research.

Conclusions and Future Prospects

Many studies have reported associations between cesarean-related gut dysbiosis and increased vulnerability to several diseases but whether these correlations are ca-

sual or causal and also what mechanisms/factors are underlying these associations remain uncertain. As a result, it remains arguable whether these disease correlations are caused by a lack of exposure to the mother's vaginal/perianal microbiota during birth or due to an increased exposure to other unsolicited bacteria, for example, from the environment, maternal skin, and so on. Also, it is indispensable to understand the indication of the delivery, particularly considering the fact that the rate of cesarean delivery is unreasonably increasing at an alarming pace worldwide. In fact, the rapidly growing worldwide prevalence of diabetes, obesity, and autoimmune and cardio-metabolic diseases over the past 2 decades has been speculated to be attributed, at least in part, to the corresponding surge in the rate of C-section deliveries. Also, the reason why these associations still remain arguable is the uncertainty of possible confounding factors inherently allied with the C-section. For example, mothers consuming a high-fat diet during gestation and the overweight and obese mothers are more likely to deliver via C-section. Also, maternal high-fat diet and the resultant obesity may affect the baby's microbiome. Hence, the independent effects of maternal diet, gestational obesity and the cesarean surgery on baby's microbiome remain indistinguishable. Furthermore, compared to vaginally born babies, cesarean-born babies generally spend a longer time at the hospital post birth and this could have several confounding effects such as higher exposure to the hospital "flora," an abrupt breast-feeding regimen during the first few days of life, different array and duration of surrounding environmental exposures, and so on on the baby's microbiota. Also, women delivering via C-section generally receive a different (and longer) regimen of antibiotics, which may influence the neonatal microbiome. Also, the spectrum and magnitude of the mode of infant gut bacterial acquisition, that is, which bacteria comes essentially from the mother and which from the environment (vertical vs. horizontal transmission), remain to be decoded completely. Notably, whether and how the gut microbiota differs between elective versus medically indicated cesarean sections also remain to be demarcated. Indeed, all-inclusive understanding of the precise routes and sources of various bacteria in the prenatal and neonatal gut would untangle some of these confusions while finding novel and effective targets for preventing gut dysbiosis and associated diseases.

The mounting evidence that the fetal life is not fundamentally devoid of environmental stimulants evidently suggests that the modulation of gestational, prenatal, and perinatal environment may prove to be effective for the

beneficial modulation of the host immunity, thereby highlighting the need for further research to survey the prospects of amending the host epigenetics during early life and also to explore whether such epigenetic modifications could also be induced not only during early life but also during later stages of life. Prospective studies that integrate dynamics and features of the maternal and infant gut microbiomes as well as other potential expounding versus confounding variables are clearly anticipated to elucidate the mechanisms driving these correlations and to discover novel and effective strategies to prevent the neonatal gut dysbiosis and related diseases.

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Disclosure Statement

The authors declare that they have no conflicts of interest to disclose.

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