2.05 Development of Microbiota - Is the Process Continuing Through Adolescence?

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2.05.1 Introduction

As a newborn takes his first breath, his epithelial surfaces become colonized by microbes. This event starts a life-long relationship between our bodies and the complex communities of microbiota (also called microbiome) living on and inside us. At different periods of life, these microbial communities show higher or lower propensity for compositional and/or functional changes. The most drastic alterations are believed to happen in the first 2–3 years of human life, when events such as breast feeding, introduction of solid foods, contact with domestic animals and environmental microbes, and the development of child immune system lead to multiple drastic changes in the human-associated microbiota (see (Munyaka et al., 2014) for a review).

The stability of microbiota after this stage varies with age. It is generally accepted that after reaching adulthood human microbiome achieves a relative level of temporal stability. While short-term day-to-day fluctuations are frequent based on, for example, the specific consumed foods (in case of gut microbiota) or environmental exposure (in case of skin microbiota), microbiome is usually resilient to such forces and maintains stable composition and functional capacity (Lozupone et al., 2012). At the same time, advanced age has been shown to lead to microbiome changes, in part due to the reduced activity of the immune system.

The question of stability of human microbiomes during child growth and development has generally been overlooked. Historically, the initial view assumed that human microbiome reaches an adult-like state by 2 or 3 years of age, and then remains stable until the old age. This view has since been challenged in a number of studies published in the past decade. One of the difficulties in achieving consensus on this subject has been the relative scarcity of studies focused on the comparison of microbiota in pre-school, school, or adolescent children to that of adults or infants. As was pointed out by Derrien and co-authors (Derrien et al., 2019), only a very small percentage of human microbiome studies included samples from these age groups (**Fig. 1**). In many cases, the studies focused on the comparison within these age groups between healthy individuals and those diagnosed with a disease.

In this chapter, we review the available reports describing potential differences of child and adolescent microbiota with that of adults or very young children. We focused on the studies that used culture-independent molecular methods to profile microbiota composition and functions. Almost all available publications profiled fecal or intestinal microbiota, likely due to its recognized importance in the development of gut diseases and cross-talk with host immune system (Lazar et al., 2018). Thus, our review will similarly focus on the gut microbiota in pre-school, school, and adolescent children. We include examples of studies unraveling the influence of different environmental factors on gut microbiota of older children, and also consider cases where little to no difference between adult and adolescent microbiota was observed.

2.05.2 Age-Related Changes in the Physiology of Intestinal Tract

What can be potential causes of the continuing changes in human microbiota past 3 years of age? To some degree, such changes can be associated with gradual alterations in the food consumption habits and changes in the child diet based on individual preferences

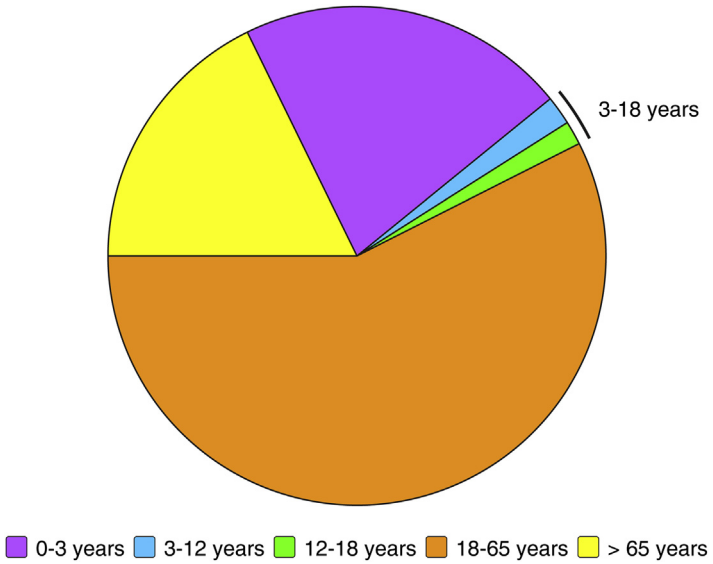

Fig. 1 Comparison of the Total Number of Gut Microbiota Samples Analyzed by Shotgun Metagenomic Sequencing, Separated by Age Groups. Based on the data tabulated in **Fig. 1** of Derrien et al. (2019), which is reused under the terms of the CC BY-NC-ND license.

or specific meal plans at schools. Physiologically, there seems to be relatively little difference in the pH and buffering capacity of different gastrointestinal tract regions between older children and adults (reviewed in (Merchant et al., 2016)). One of the more noticeable changes involves the maturation of the mucosal immune system. The number and the size of Peyer's patches in small intestine increase through the childhood until puberty, and the number of lymphoid follicles in the colon of adolescent children was found to be about 2-fold higher than that in adults (Dukes and Bussey 1926). Johnson and colleagues (Johnson et al., 2001) revealed a gradual increase throughout the child development in the expression of intestinal metabolic enzymes such as cytochrome P450. The developing host can also continue to affect microbiota composition in order, for example, to support colonization by microbes well adapted to the adolescent and adult-like diets.

2.05.3 Microbial Abundance Gradients Were Observed During Childhood in a Healthy Population From an Indian Village

Balamurugan and colleagues (Balamurugan et al., 2008) recruited 130 healthy children and 30 healthy adults all living in a single rural area in southern India. The age of children spanned between 2 and 17 years of age; adults had ages between 28 and 50 years. Quantitative polymerase chain reaction with primers targeting 16S rRNA gene of the select microbial taxa was used to assess the differences among participants' fecal microbiota. Abundance of *Lactobacillus acidophilus* declined monotonously with age from 2 years till and including adulthood (**Fig. 2**). The total bifidobacteria reduced slowly with age of children and adolescents before diminishing greatly in adulthood. *Bacteroides* displayed an opposite trend. The abundances of *Faecalibacterium prausnitzii* and *Eubacterium rectale* peaked around 8–9 and 14–17 years of age, and their abundance diminished afterward.

Based on the obtained data, the authors concluded that there was a "continuing and gradual change in fecal bacterial flora beyond early childhood and this change continues into adolescence and adulthood" (Balamurugan et al., 2008).

2.05.4 Distal Gut Microbiota of Adolescent Children Was Different From That of Adults in the Healthy US Cohorts

In 2011, we published one of the first direct high-throughput comparisons of healthy gut microbiota between pre- and adolescent children and adults (Agans et al., 2011). A total of 22 children with an age range of 11–18 years and 10 adults (age range: 22–61 years) were recruited in the Mid-West region of United States, and the composition of their fecal microbiome was interrogated with Affymetrix-based Microbiota Array (Paliy et al., 2009). There was no significant difference in the community richness between groups (defined as the number of detected species). However, based on the principal component ordination analysis (PCA), overall structure of fecal microbiomes differed between children and adults with statistical significance (see **Fig. 1** in the original publication; $p < 0.0001$ as defined with Davies-Bouldin index). The distribution of samples along the principal component 1 axis correlated negatively with subject age, which was interpreted as an indication that age was one of the main factors differentiating fecal samples in the PCA space. Comparing the relative abundances of different bacterial classes, adolescents harbored more Actinobacteria, Gammaproteobacteria, and Deltaproteobacteria, whereas adults had higher levels of Betaproteobacteria (primarily *Sutterella*) and Clostridia (**Fig. 3**). Notably, the abundance of actinobacterial genus *Bifidobacterium*, which is highly prevalent in young

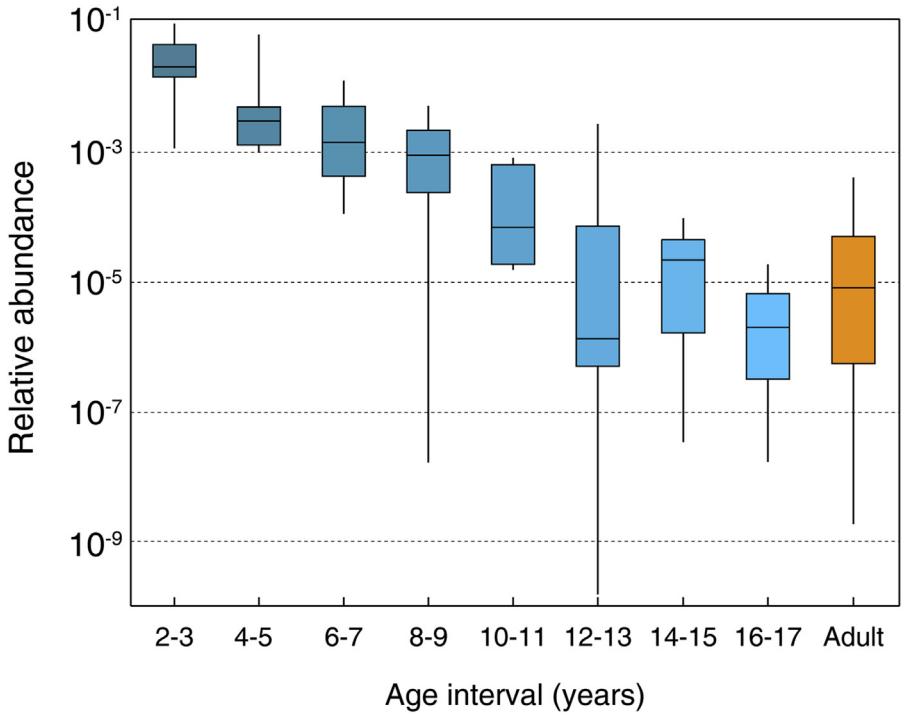

Fig. 2 Proportion of Total *Lactobacillus acidophilus* in Fecal Samples From Healthy Indian Volunteers of Different Age Groups. Each box shows the median (middle line), interquartile range (box), and data range (whiskers) of values among the group members. Y axis is plotted on logarithmic scale. Proportions of *L. acidophilus* in samples from subjects with ages 2–3, 4–5, 6–7, and 8–9 were statistically significantly different from those in samples from age groups 12–13, 14–15, 16–17, and the adult group. The figure is based on the data originally depicted in **Fig. 2**B of Balamurugan et al. (2008), and is reused with authors' permission.

children, was 67% higher in older children than in adults (9.0% vs 5.4% relative abundance, respectively). The level of bifidobacteria correlated negatively with age (Spearman's rank Rs = −0.29, $p = 0.03$). This difference was due to an increased relative abundance of genus members rather than their presence or absence – the number of *Bifidobacterium* species detected per sample was the same for both groups (Agans et al., 2011). Among the top dozen most abundant genera, only *Bifidobacterium* and *Clostridium* displayed a statistically significant difference between adolescent and adult groups. However, statistically significant differences between these cohorts were observed for several other, lower abundance genera including *Prevotella*, Butyrivibrio, *Sutterella* (all higher in adults), *Enterobacter*, *Limnobacter*, and *Turicibacter* (all higher in teenagers).

The authors argued that their findings are "more consistent with the model where levels of bifidobacteria in children decrease gradually between 2 and 18 years of life until reaching stable levels in the early adulthood, rather than with the model that assumes that the levels of these bacteria decline quickly after weaning" (Agans et al., 2011).

2.05.5 Significant Alterations Are Found in Human Gut Microbiota With Age and Across Populations

Fecal microbiota of 531 subjects from Venezuela, Malawi, and the United States was profiled in a large-scale study by Yatsunenko and co-workers (Yatsunenko et al., 2012). The subject cohorts comprised Amazonian Amerindians from Venezuela, four rural African communities of Malawi, and urban population in the US. Age range for enrolled subjects was between 0 and 83 years. High-throughput sequencing of the V4 variable region of 16S ribosomal RNA gene was used to estimate community structure. Subject age correlated strongly with the distribution of samples in the reduced ordination space, as evidenced by the age gradient along principal component 1 of the UniFrac-based PCoA plot (see Supplementary Figure S2a in the original publication). While the most drastic changes occurred within the first 3 years of life, the microbial communities continued to gradually shift through the childhood (see **Fig. 2**A in the original publication). In all three populations, interpersonal variation was significantly greater among children than among adults. Overall, the geography/population lifestyle was the most significant predictor of community structure. Fecal microbiota of US subjects clustered separately from that of Amerindians and Malawians. Interestingly, the diversity of fecal microbiota in US individuals was much lower than in the other two cohorts (Yatsunenko et al., 2012).

Using a subset of babies, children, and adults, authors determined which bacterial taxa changed with increasing age. Abundances of *Bifidobacterium longum*, *Bifidobacterium breve*, *Streptococcus thermophilus*, and several strains of enteric bacteria correlated negatively with age in all three populations. The strongest positive influence of age was observed for the abundances of *Eubacterium* species,

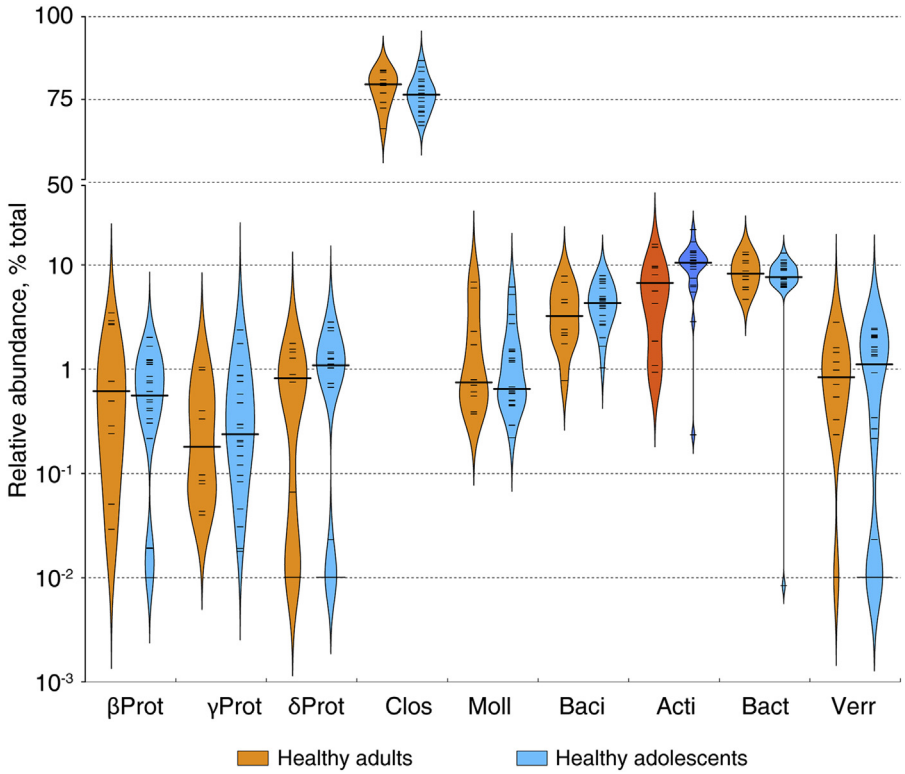

Fig. 3 Distribution of the Relative Abundances of the Nine Most Prevalent Bacterial Classes Among Fecal Samples Obtained From Healthy US Pre- and Adolescent Children and US Adults. Data are shown in bean plots; each bean depicts local density distribution of class abundances among all samples in the group. Bold line shows the average abundance within each group, and individual sample abundances are shown with dashes. Y-axis has a linear scale between 50% and 100% relative abundance and logarithmic scale between 50% and 0.001%. βProt - Betaproteobacteria, γProt - Gammaproteobacteria, δProt - Deltaproteobacteria, Clos - Clostridia, Moll - Mollicutes, Baci - Bacilli, Acti - Actinobacteria, Bact - Bacteroidia, Verr - Verrucomicrobiae. Bean plots for class Actinobacteria that displayed the most striking difference between adult and adolescent samples are highlighted. The figure is based on the data originally reported by Agans et al. (2011), that was used with authors' permission.

Faecalibacterium prausnitzii, *Ruminococcus bromii*, and *Victivallis vadensis*. Many differences were found in the microbiome composition between US and other two groups, including prevalence of *Prevotella* in non-US adults and higher abundance of *Bacteroides* in US subjects (Yatsunenko et al., 2012). Authors argued that the observed differences in gut microbiota can be related to the differences in consumed diets. While typical US diet is rich in animal proteins and fats, diets in Malawian and Amerindian populations are dominated by corn and cassava. Indeed, the functional gene repertoires in these communities reflected the differences observed in carnivorous versus herbivorous mammals (Muegge et al., 2011). For example, glutamate synthase was more prevalent in Malawian and Amerindian adult microbiomes; this enzyme is also higher in herbivorous mammalian microbiomes. In contrast, the degradation of glutamine was overrepresented in US as well as carnivorous mammalian microbiomes.

Overall, subject's age and consumed diet were the two most prominent determinants of the fecal microbiota structure and functional capacity (Yatsunenko et al., 2012).

2.05.6 The Diversity of Antibiotic Resistance Genes Encoded by Human Gut Microbiota Is Age Related

Lu and colleagues (Lu et al., 2014) studied the distribution and diversity of antibiotic resistance (AR) genes that are encoded in the genomes of human gut microbiota. Fecal samples from 124 healthy volunteers of four different age groups - pre-school-aged children (CH, 3–5 years old), school-aged children (SC, 10–11 years old), high school students (HSS, 15–17 years old), and adults (AD, 26–55 years old) - were collected in Hangzhou, China. None of the participants were knowingly exposed to any antibiotics for at least 3 months prior to sample collection. Metagenomic DNA was isolated and subsequently hybridized to the microarray chip containing probes targeting 2915 antibiotic resistance genes. A total of 80 different antibiotic resistance gene types were recovered from the gut microbiota of these 124 individuals. Among the detected genes were those conferring protection against aminoglycosides, beta-lactams such as penicillin, amphenicols, trimethoprim, sulfonamides, and tetracyclines (Lu et al., 2014). The diversity of AR gene types increased with age: 25, 37, 58 and 72 gene types were identified in the CH, SC, HSS and AD groups, respectively (**Fig. 4**). The average number of gene types in an individual was 8, 14, 15, and 24, among CH, SC, HSS and AD groups, respectively. Principal coordinate analysis of the antibiotic resistance dataset revealed that the samples were clustered in the PCoA space consistent with

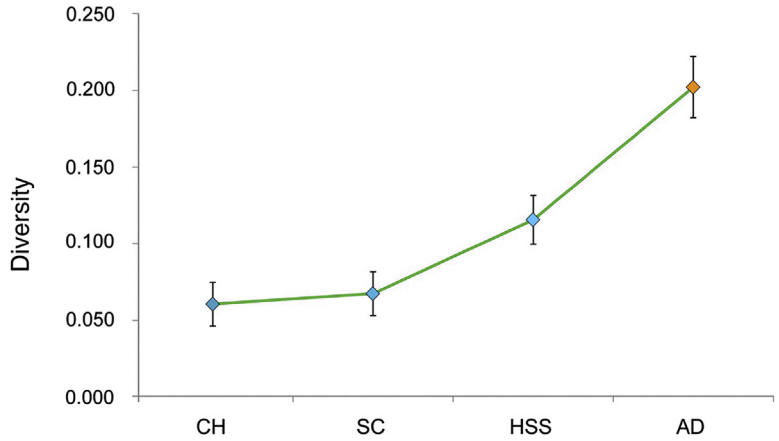

Fig. 4 Diversity of Microbiota-Encoded Antibiotic Resistance Genes Among Fecal Samples Collected From Healthy Chinese Pre-school-aged Children (CH), School-Aged Children (SC), High School Students (HSS), and Adults (AD). Diversity calculations are based on the Gini-Simpson index. This figure is based on the plot presented in **Fig. 1**B in Lu et al. (2014), which is reused under the terms of the CC BY 3.0 license.

group identity (see **Fig. 2**A in the original publication). The variability in the AR profiles was largest in the adult group, which was potentially attributed to the differences in life exposure to different antibiotics. Authors concluded that "the antibiotic resistance gene diversity in the human gut microbiota was increasingly complex as individuals aged and that diversity likely accumulated from childhood to adulthood".

2.05.7 Structure and Functions of Gut Microbiomes From Healthy School-Age Children Show Features That Distinguished Them From Those of Healthy Adults

The gut microbiota of 37 healthy school-age children (age range 7–12) was profiled by Hollister and co-workers (Hollister et al., 2015). Both 16S rRNA amplicon sequencing and shotgun metagenomic sequencing were employed, and these data were contrasted against a comparable set available from the Human Microbiome Project for healthy adults. While sex, race, and body mass index had either no or only marginal influence on the diversity and structure of gut microbiota across the full dataset, age group (children or adults) was associated with significant differences. The average healthy child's gut community contained significantly lower abundances of *Bacteroides* and *Parabacteroides* and significantly greater abundances of *Bifidobacterium*, *Faecalibacterium*, *Roseburia*, and *Dialister* (Hollister et al., 2015). The differences were sufficiently large to cause a partial, but statistically significant separation of child and adult sample groups in reduced ordination space (**Fig. 5**A). This differentiation of child and adult profiles was evident regardless of the dissimilarity measure used. Abundances of operational taxonomic units (OTUs) were used to build a Random Forest based discrimination model which correctly classified 85% of samples (68 out of 80). Among the top discriminatory OTUs were members of genera *Bifidobacterium*, *Faecalibacterium*, and *Bacteroides*.

At the functional level, many differences were revealed between adolescent and adult microbiomes. In fact, more than 1500 KEGG ortholog groups (KO) differed between healthy children and adults (Hollister et al., 2015). Pathways with higher prevalence in the gut of healthy children included folate and cobalamin biosynthesis, metabolism of the amino acids tyrosine, lysine, cysteine, and methionine, and porphyrin and methane metabolism. Conversely, adult gut communities were significantly enriched in genes involved in oxidative phosphorylation, TCA cycle, lipopolysaccharide biosynthesis, flagellar assembly, and steroid hormone biosynthesis (**Fig. 5**B and C).

Authors concluded that "childhood appears to represent a unique transitional stage with respect to the gut microbiome" and that "although the healthy pediatric gut microbiome harbors several adult-like features, it also retains many of its own distinct compositional and functional qualities" (Hollister et al., 2015).

2.05.8 Child Microbiota Continues to Evolve Into Adulthood in a Healthy Chinese Population

More than 1000 healthy Chinese subjects from Jiangsu province with the ages from 3 to over 100 years were enrolled into a gut microbiota study by Bian et al. (Bian et al., 2017). All participants were divided by age into eight groups: kindergarten students, ages 3–6; primary school students, ages 8–12; middle school students, ages 13–14; college students, ages 19–24; young adults (soldiers and police recruits), ages 19–24; middle-aged adults, ages 30–50; elderly, ages 60–79; and centenarians, at least 94 years old. Fresh fecal samples were collected from each individual, and V4 region of 16S rRNA gene was sequenced on Illumina MySeq platform.

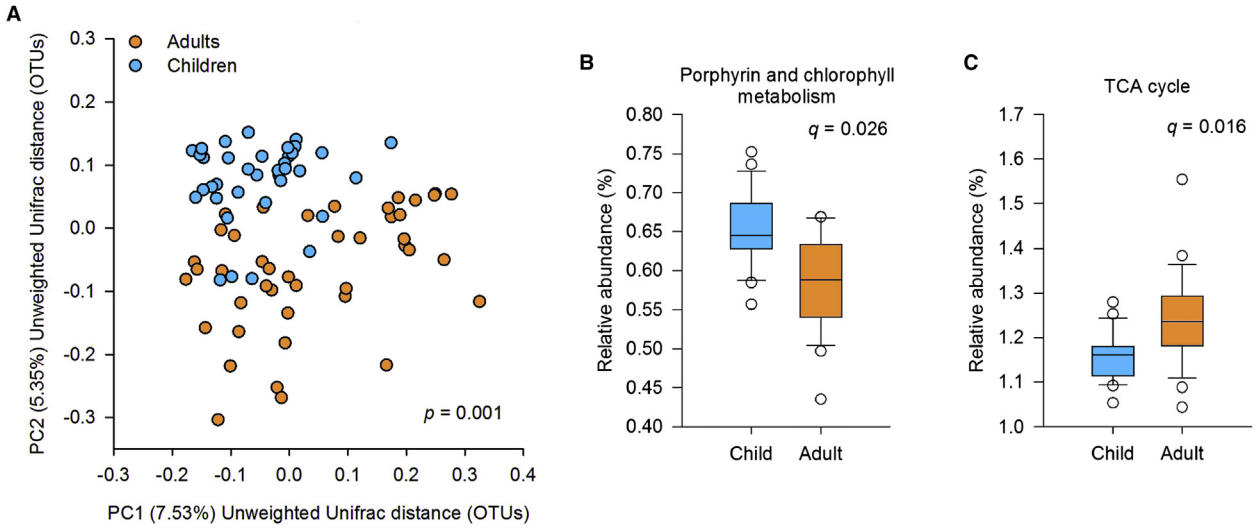

Fig. 5 Differences in the Composition and Function of Healthy US School-Age Children and Adults. Panel A displays the separation of child and adult samples in the principal coordinate space based on their microbiota composition. Unweighted UniFrac distance was used to estimate sample (dis)similarity. Percent variance attributed to each axis is indicated in parenthesis. P value represents the significance of sample separation between groups in PCoA space. Panels B and C show the distribution of relative abundances of the functional genes encoding for porphyrin and chlorophyll metabolism (B) and for TCA cycle enzymes (C) among child and adult fecal samples. Each box shows the median (middle line), interquartile range (box), and data range (whiskers) of values among the group members. Outliers are represented by individual circles. Q value represents the false discovery rate based significance of the observed differences between groups. This figure is based on plots shown in **Fig. 4**B and C, and S3a of Hollister et al. (2015). The original plots are reused under the term of the CC BY 4.0 license.

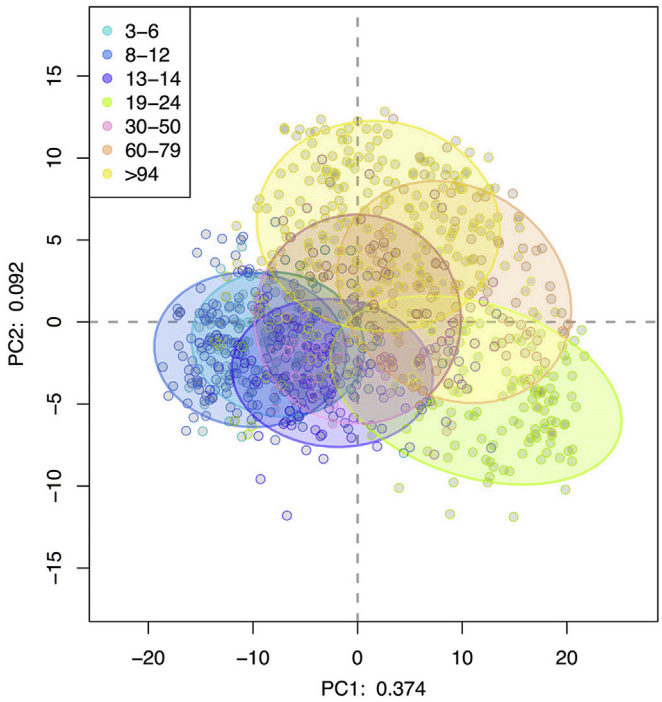

Fig. 6 Principal Components Analysis (PCA) of the Microbiota Composition of Fecal Samples From Healthy Chinese Population. Only operational taxonomic units with large effect size were included in the PCA analysis. The fraction of the total variance attributed to each axis is indicated in each axis title. Samples are colored by the age of the donor as shown in figure legend; group clouds represent 75% confidence interval. The figure is based on the PCA plot originally presented by Bian et al. (2017), and is reused under the terms of the CC BY 4.0 license.

The ordination analysis of the community structure revealed that samples could be separated in the PCA space according to age group, though group boundaries overlapped in most cases (Fig. 6). Notably, a gradual progression of sample group centroids was observed for groups with ages 8–12, 13–14, and 19–24. OTUs from genus *Bifidobacterim* were associated with child samples, whereas older age groups were related to the presence of *Faecalibacterium* and *Bacteroides* (Bian et al., 2017).

Authors then examined any potential associations of the abundances of several notable genera with age (see Fig. 2 in the original publication). These analyses confirmed ordination results and revealed gradual reduction in fecal *Bifidobacterium* abundance with subject's age. At the same time, abundance of *Faecalibacterium* had a peak prevalence in the middle age period (20–60), after which point its abundance declined. The abundance of members of genera *Prevotella* and *Bacteroides* increased sharply in children between ages 3 and 18 and reached its peak at 20 years of age. Authors also detected a gradual increase in community diversity between ages of 2 and 14, though a lot of variability was evident at each age among different subjects. Among the profiled samples, community diversity declined significantly at age 20, which was followed by a quick recovery.

Authors speculated that "there are large differences in relative abundance of the OTUs of many genera between subjects aged 19–24 and younger subjects. If we take the view that this is a cohort effect, we could conclude that members of multiple genera form a minimum or maximum relative abundance near age 20" (see Fig. 6). "This suggests that a change in lifestyle (e.g., leaving home for university or jobs) or physiology (e.g., levels of sex steroid hormones) in the post-teen years is an important determinant of the observed gut microbiota" (Bian et al., 2017).

2.05.9 Healthy Korean Adolescents Harbor Different Gut Microbial Community Than Healthy Adults

In a study of Kim and colleagues (Kim et al., 2018), 67 healthy Korean adolescents between ages 13–16 were recruited, and their microbiome was compared to (i) a large dataset obtained previously for healthy Korean adults ($n = 1463$) and to (ii) healthy adult samples from the Human Microbiome Project. Both adult cohorts housed more diverse gut microbiota than Korean adolescents. Genera *Bacteroides* and *Prevotella* and phylum Bacteroidetes were elevated in adolescent samples. Abundance of *Faecalibacterium* was higher in Korean adults. Using PICRUSt analysis, authors estimated functional capacities of different communities and found differential abundances of secondary bile acid synthesis genes between adolescent and adult populations. Many other KEGG pathways were predicted to be differentially encoded between the adolescent and adult gut microbiota (Kim et al., 2018). Note, however, that different 16S rRNA gene variable regions were profiled in different cohorts, and different sequencing platforms were utilized, which likely accounts for some of the observed differences.

2.05.10 Other Studies Also Reveal Changes in Gut Microbiota Composition in Pre-school and School Age Children in Comparison With Other Age Groups

In addition to the studies we highlighted above, a number of other projects have been described that considered potential uniqueness of microbial communities in older children.

- Among 60 Finnish 7-year old children, the mode of their delivery (vaginal or caesarian) affected the ratio of *Bifidobacterium* and Clostridia in their stools: clostridial numbers in vaginally born children seven years after delivery were significantly higher than in caesarean born children (Salminen et al., 2004).
- The prevalence of methanogenic Archaea in the stool of adolescent children was lower than that in the stool of adults (Vanderhaeghen et al., 2015). The presence of Methanobacteriales correlated with the occurrence of bacterial taxonomic groups associated with carbohydrate degradation to hydrogen and the formation of formate.
- In a cohort of 195 Malian children (age range 0.25–17 years) and young adults (18–25 years), overall fecal microbial diversity increased with age, and age was the dominant factor influencing distribution of samples in the ordination space (Yooseph et al., 2015). Significant association between microbiota composition and the prospective risk of infection with *Plasmodium falciparum* was detected; the infection rate was higher in 12–17 year old adolescents and young adults.
- Prevalence of xenobiotic metabolizing enzymes in the gut metagenomes of 397 subjects from eight different countries was investigated by Das and co-workers (Das et al., 2016). Children (age range: 0–10 years) contained the lowest total abundance of these enzymes. This trend was followed by a sudden increase in older children and young adults (10–30 years old), and subsequent saturation in abundance was observed later in life.
- HITChip phylogenetic microarray was used to quantify microbiota in children between 1–5 years of age (Cheng et al., 2016). The bacterial diversity and evenness remained lower in 5 year old children compared with adults. Actinobacteria, Bacilli, and *Clostridium* cluster IV showed comparable abundances among children that differed from their levels in adults, whereas some other groups such as *Clostridium* cluster III were converting to adult-like profiles in 5 year olds.
- In a large-scale study of 367 healthy Japanese individuals spanning age range of 0–104 years, gut microbiota composition continued to be altered during pre-adolescent, adolescent, and early adulthood (Odamaki et al., 2016). This included an overall gradual increase in microbiota alpha diversity, the reduction in *Bifidobacterium* abundance, and the concomitant increase in *Bacteroides* and Lachnospiraceae.

- In a follow-up study that focused on the prevalence of *Bifidobacterium* species, authors revealed that different *Bifidobacterium* species were dominant in different age groups (Kato et al., 2017). Specifically, *B. breve* was detected mostly in children under 3 years of age, whereas *B. adolescentis* and *B. catenulatum* groups were dominant after weaning.
- Age was the most significant contributing factor to fecal microbiota diversity and community composition in a cohort of 75 healthy 2-to-9 year old US children (Herman et al., 2020). The relative abundance of several bacterial taxa was linked to consumption of particular food groups and/or nutrients; for example, *Lachnospira* abundance correlated positively with the consumption of fruits and fiber.

It is important to recognize that not all studies found noticeable differences between microbial communities of adults and older children. Two examples are provided below.

- A large-scale analysis of fecal samples from 903 children was conducted as part of the Environmental Determinants of Diabetes in the Young (TEDDY) study (Stewart et al., 2018). Children between 3 months and 4 years of age were profiled who were at risk for developing islet autoimmunity or type I diabetes. Both 16S rRNA amplicon and metagenomic sequencing of fecal genomic DNA were employed. Using linear mixed-effects modeling of the abundances of the top five phyla and Shannon's diversity index, authors defined three phases of microbiota progression: a developmental phase (months 3–14), a transitional phase (months 15–30), and a stable phase ($\geq$31 months). While many changes were observed in the first two stages, the prevalence of the most abundant phyla and the Shannon diversity index were steady during the stable phase (see **Fig. 1**C in the original publication). Assessing metagenomic data, while microbiota community was generally most stable after 2.5 years of subject age, more transitions between clusters were detected (see Extended **Fig. 2**C in the original publication). Many factors affected microbiota composition in the first year of life including birth mode, breast feeding, geographical location, and the use of probiotic supplements. However, these factors no longer significantly distinguished microbiomes of 3-to-4 year old children (Stewart et al., 2018). Authors "considered the first year of life as developmental, the second year of life as transitional, and from year three of life the microbiome stabilized".
- Stool samples from 281 children of 6–9 years of age (mean age 7.3 years) enrolled in the KOALA Birth Cohort Study (KOALA is an acronym in Dutch for "child, parents and health: lifestyle and genetic constitution") were profiled by Zhong and co-workers (Zhong et al., 2019). All samples were analyzed by shotgun metagenomic sequencing, and this dataset was compared to the metagenomic dataset available from 62 healthy Dutch adults. Community diversity as measured by Shannon's alpha diversity index was similar between child and adult fecal microbiota, and samples could not be separated according to age group in the ordination PCA space (see **Fig. 1** in the original publication). Nevertheless, Dutch children revealed a statistically significant enrichment of genus *Bifidobacterium* in their stools compared to adults. Further comparisons showed that children were enriched in bacteria from the phylum Bacteroidetes including *Bacteroides* and *Prevotella*, while Firmicutes assigned to genera *Eubacterium*, *Clostridium*, *Dorea*, and *Coprococcus* were more abundant in adults (Zhong et al., 2019). Similar to the analysis originally conducted in adults (Arumugam et al., 2011), three distinct enterotypes were also detected in Dutch children, defined by high abundance of genera *Bacteroides* (enterotype E1), *Prevotella* (E2), and *Bifidobacterium* (E3). Microbiota of E1 enterotype was adult-like, whereas E3 still retained simpler-structured composition more typical of younger children. Further differences among enterotypes were observed among the metabolic functions encoded by each community. In contrast to the Stewart et al. study described above, authors found that several factors, including breastfeeding duration in early life and plant-based food intake, correlated significantly with the gut microbiota composition in school-age children.

2.05.11 Environmental and Health Differences Among Children Lead to Different Microbiota Profiles

So far, we focused our description on the consideration of any differences in the microbiome of older children in comparison to adults and babies. There is also a growing body of studies which assessed potential differences in microbiota composition and/or functions among several groups of similar-age children. Below we highlight examples of such studies.

- A number of studies compared microbiota of children living in different geographical regions. For example, healthy Bangladeshi children, aged 9–14, harbored a significantly more diverse gut microbiota compared with healthy US children of comparable age (Lin et al., 2013). The microbiome of Bangladeshi children was enriched in *Prevotella*, *Butyrivibrio*, and *Oscillospira* and was lower in *Bacteroides* relative to US children.
  - We previously contrasted distal gut microbiota of US and Egyptian pre- and adolescents (Shankar et al., 2017). Egyptian children housed significantly more members of *Prevotella*, whereas *Bacteroides* was more abundant in US children. Abundances of *Faecalibacterium* and *Akkermansia*, two genera with recognized anti-inflammatory effects, were also higher in US subjects. The intestinal environment of Egyptians was characterized by higher levels of short-chain fatty acids, a higher prevalence of microbial polysaccharide degradation-encoding genes, and a higher proportion of several polysaccharide-degrading genera, all consistent with the plant-rich Mediterranean diet that Egyptians consumed. Microbiota of US children had higher prevalence of amino acid and lipid metabolism associated functions, likely related to the consumption of protein- and fat-rich diet in US.
- Dietary patterns were also found to influence gut microbiota composition in a cohort of 4–8 year old healthy US children (Berding et al., 2018). Diet rich in fish, refined carbs, vegetables, and meat was associated with the higher relative abundance of

Bacteroides and *Ruminococcus*, and the lower abundance of *Bifidobacterium*, *Prevotella*, and *Blautia*. Alternative diet, high in starchy foods, grain, and dairy, was associated with the higher relative abundance of *Phascolarctobacterium* and the lower abundance of *Dorea* and *Eubacterium*.

- Effects of probiotic supplementation on the gut microbiota of healthy Japanese school children (mean age 7.7 years) included significant increases in the population levels of *Bifidobacterium* and *Lactobacillus* and simultaneous decrease in the levels of Enterobacteriaceae, *Staphylococcus* and *Clostridium perfringens* (Wang et al., 2015).
- In a meta-analysis spanning 1020 healthy individuals drawn from 23 populations and six published studies, the abundances of major bacterial phyla were assessed as a function of altitude the subjects live at (Suzuki and Worobey 2014). Authors found a positive correlation between Firmicutes and latitude and a negative correlation between Bacteroidetes and latitude; this relationship did not depend on age, sex, or detection method used.
- Fecal microbiota from 208 Tibetans from six different locations, which varied in altitude from 2800 m to 4500 m above the sea level, were analyzed by Illumina sequencing by Lan et al. (Lan et al., 2017). Constrained ordination analysis of the dataset revealed that Ruminococcaceae, *Prevotella*, and Lachnospiraceae correlated negatively with altitude, while *Faecalibacterium*, *Bacteroides*, and *Bifidobacterium* showed positive association with altitude, BMI, and age.
- Sjödin and colleagues collected stool samples from 93 children at 4, 6, 13 months, and 8 years of age at a risk of developing allergic diseases (Sjödin et al., 2019). Analysis of the fecal microbiota revealed consistent underrepresentation of *Bacteroides*, *Prevotella*, and *Coprococcus* in allergic compared to nonallergic children from infancy to school age. The gut microbiota of the allergic 8-year-olds was enriched in *Bifidobacterium* and depleted of *Lactobacillus*, *Enterococcus*, and *Lachnospira*.
- Intestinal microbiota composition and intestinal inflammation were evaluated in children and adolescents diagnosed with cystic fibrosis (CF) and compared to those in healthy controls (de Freitas et al., 2018). Expectedly, intestinal inflammation was higher in subjects with CF. *Bacteroides* spp., *Eubacterium rectale*, and *Faecalibacterium prausnitzii* significantly decreased in the gut of CF group compared to the controls, whereas the levels of *Clostridium difficile*, *Escherichia coli*, and *Pseudomonas aeruginosa* significantly increased.
- Several studies investigated the associations of gut microbiota with childhood obesity. Comparison of obese and normal weight 6–16 year old Italian children revealed that obesity was associated with an altered gut microbiota including elevated levels of Firmicutes and lower levels of Bacteroidetes (Riva et al., 2017). Abundance of *Bacteroides* correlated positively with the body mass index (BMI) score.
  - Higher BMI was associated with depletion of Actinobacteria and increase in Proteobacteria in the cohort of 267 children aged 7–18 years from the American Gut Project (Bai et al., 2019). BMI had significantly higher effect on microbiota composition compared with diet and exercise.
  - Finally, diversity of gut microbiota diminished with increasing weight among 6–11 year old obese and normal weight Chinese children (Chen et al., 2020). The differences in microbiome composition allowed samples to be separated in the ordination space. *Faecalibacterium*, *Phascolarctobacterium*, *Lachnospira*, *Megamonas*, and *Haemophilus* were significantly more abundant in the obese group; the proportions of *Oscillospira* and *Dialister* were in turn lower.
- Pulikkan et al. detected a substantial dysbiosis in the gut microbiome of children diagnosed with autism spectrum disorder (ASD) (Pulikkan et al., 2018). Children with ASD harbored more Lactobacillaceae, Bifidobacteriaceae, and Veillonellaceae, whereas the gut microbiome of healthy children was dominated by the family Prevotellaceae. Among these taxa, species of genus *Lactobacillus* showed a particularly strong association with ASD.

2.05.12 Finding Common Trends Among Different Studies

Recently, a detailed meta-analysis of the published reports on the composition of child gut microbiota was conducted by Deering and colleagues (Deering et al., 2019). The authors searched the publication databases for the terms "healthy preadolescent children" and "gut microbiome" and compiled a list of 42 articles, including many of those we mentioned above. While there was a limited congruency among different studies, some trends have been identified. Geographic location influenced microbial community richness and metabolic potential: subjects living in non-westernized countries tended to have more diverse gut microbiota which produced higher amounts of short chain fatty acids (Deering et al., 2019). Considering differences among children based on their age, authors revealed a general trend for *Bacteroides*, *Prevotella*, and *Dialister* to increase its abundance with child development. The opposite effect was noticeable for the abundances of *Bifidobacterium*, *Ruminococcus*, and *Streptococcus*. *Bifidobacterium* and *Streptococcus* are well recognized early colonizers of the human gut, which were previously thought to significantly reduce their abundance by 2 or 3 years of age. The trend of gradually decreasing *Bifidobacterium* abundance in childhood and adolescence has indeed been reported in several studies we described above. Deering and co-workers mentioned that "despite the quantity of research in the area, the researchers identified no obvious reason for the decrease in relative proportions of bifidobacteria from early childhood into puberty and may be inversely related to proliferation of other bacteria".

2.05.13 Summary

There is now ample evidence made available by the plethora of recent molecular high-throughput studies of human microbiota in childhood and adolescence to refute the previously held belief that human microbiota largely matures by 2–3 years of age and at that point resembles the microbiome of a healthy adult. It appears that gradual changes in relative composition and encoded functions of microbial communities take place throughout human development, and the human microbiota might reach relative stability in early adulthood. We are yet to uncover the forces that drive such longitudinal changes. These might include the gradual shifts in each person's dietary habits, hormonal changes during child development and puberty, evolving host-microbial signaling, and continuing exposure to novel environmental agents and sources of microbes.

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