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Minireview

The human gut microbiome in health: establishment and resilience of microbiota over a lifetime

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Summary

With technological advances in culture-independent molecular methods, we are uncovering a new facet of our natural history by accounting for the vast diversity of microbial life which colonizes the human body. The human microbiome contributes functional genes and metabolites which affect human physiology and are, therefore, considered an important factor for maintaining health. Much has been described in the past decade based primarily on 16S rRNA gene amplicon sequencing regarding the diversity, structure, stability and dynamics of human microbiota in their various body habitats, most notably within the gastrointestinal tract (GIT). Relatively high levels of variation have been described across different stages of life and geographical locations for the GIT microbiome. These observations may prove helpful for the future contextualization of patterns in other body habitats especially in relation to identifying generalizable trends over human lifetime. Given the large degree of complexity and variability, a key challenge will be how to define baseline healthy microbiomes and how to identify features which reflect deviations therefrom in the future. In this context, metagenomics and functional omics will likely play a central role as they will allow resolution of microbiome-conferred functionalities associated with health. Such information will be vital for formulating therapeutic interventions aimed at managing microbiota-mediated health particularly in the GIT over the course of a human lifetime.

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Introduction

Technological advances in culture-independent molecular methods are allowing us to uncover a new facet of our natural history by accounting for the vast diversity of microbial life, which colonizes the human body. Human beings are now more than ever regarded as microbial ecosystems, comprising bacteria, archaea, eukaryotes and viruses with whom we have coevolved and which colonize different body habitats. The human microbiome contributes essential functionalities to human physiology and is considered essential for the maintenance of human health (Rooks *et al.*, 2014). As the microbiota in certain body habitats, e.g. the gastrointestinal tract (GIT), are temporally stable over the short- and medium-term (Voigt *et al.*, 2015), the definition of microbiome attributes associated with general host characteristics, e.g. health, should be possible. However, microbial community compositions vary over human lifetimes and geographies (Yatsunenko *et al.*, 2012). Furthermore, relatively large intraindividual differences exist between distinct body sites and considerably less but notable interindividual variation is apparent even among healthy individuals for a given body site (Grice *et al.*, 2009; Franzosa *et al.*, 2015; Voigt *et al.*, 2015). Consequently, it may be difficult to define site-specific baseline microbiomes associated with human health especially at lower taxonomic ranks (such as at the genus, species and/or strain levels). This restriction is in part due to the limited resolution afforded by 16S rRNA gene amplicon sequencing which presently is the method of choice in most studies. The structural differences which are typically resolved at higher taxonomic ranks (such as at the phylum, class and/or order levels) may not be very meaningful as many distinct taxa are typically regrouped, thereby confounding linkages between specific microbial community structures and health status. Therefore, future studies encompassing metagenomics and functional omics will allow much deeper insights into the structural and functional complements of microbiota associated with health. Nonetheless, individual body sites exhibit certain broad features associated with health, that are resolvable using 16S rRNA gene-based surveys, which include specific distributions of microbial phyla, diversity and relative stability over time (Fig. 1A).

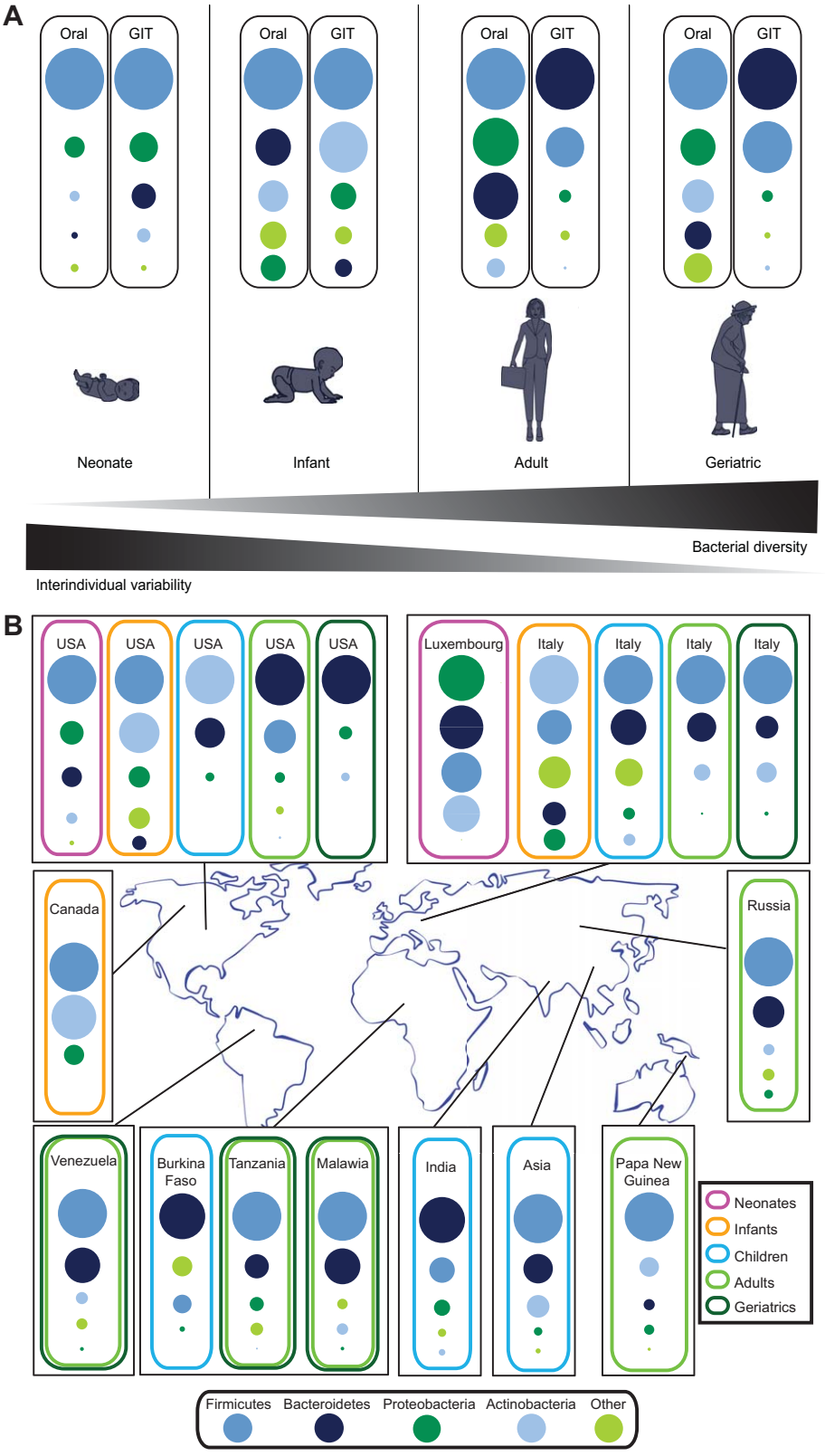

Fig. 1. Characteristics of the 'healthy' human microbiome. A. The 'healthy' microbiome of individual body sites throughout human lifetime. B. The 'healthy' gastrointestinal tract microbiome across geographies. Dots are weighted according to the overall abundance of distinct phyla. Additional information regarding the studies, based on which Fig. 1 was devised, are listed in Table S1.

The human GIT microbiome has been the major focus of studies as it contains the vast majority of microbial biomass (Eckburg *et al.*, 2005) and can relatively easily be sampled by collection of fecal material. Additionally, it is intimately involved in digestion, metabolite production, cross-talk with the immune system (Flint *et al.*, 2012), and has been implicated in numerous disease processes (Kinross *et al.*, 2011). The human GIT microbiome is dominated by four main bacterial phyla including Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria (Ley *et al.*, 2008). Given their dominance, the Bacteroidetes and Firmicutes have probably received the most attention with respect to the microbial ecology of the GIT. While the Firmicutes are primarily associated with energy harvest from food (Turnbaugh *et al.*, 2006), the Bacteroidetes are linked with several health benefits due to their capacity to degrade complex sugars and proteins into metabolizable short chain fatty acids (SCFAs) (Ley *et al.*, 2006).

Based on recent surveys, it is apparent that the human GIT microbiome changes significantly over human lifetime and that age-specific differences may be key to understanding microbiome-mediated effects on health. Although other body habitats are distinct in their microbiota compositions from the GIT, they remain much less explored with regards to differences across age and geography. Consequently, we use here the human GIT microbiome as a template for discussing the development and changes of the human microbiome over a lifetime while accounting for biogeography, host genetics, diet and other environmental influences. Although the microbial community compositions in other body habitats, e.g. the skin, are shaped by combinations of factors distinct from those in the GIT, observations in the GIT microbiome with respect to colonization, succession and dynamics may prove helpful for the future contextualization of patterns observed in other body habitats in the context of human health. In particular, the refinement of our understanding of normal variation within microbial communities will further our knowledge of their structure and function and allow us to identify key features which reflect deviations therefrom, thereby facilitating the design of advanced therapeutics aimed at managing microbiota-mediated health over the course of life.

Development and succession of the GIT microbiome over the human lifetime

In order to understand how the GIT microbiome is involved in human health, we need to understand how the microbiome develops and evolves with age. The GIT microbiome is dynamic over a human lifetime (Fig. 1A); colonization may begin *in utero*, and the undifferentiated, low diversity GIT microbiome at birth proceeds through various developmental stages with associated changes in diversity, structure and functional gene repertoires (Yatsunenko *et al.*, 2012; Hollister *et al.*, 2015).

First exposures: in utero *colonization*

Until recently, it was assumed that the fetal environment is sterile and that microbial colonization begins with birth. However, with the advent of culture-independent molecular methods, recent evidence has called this assumption into question. The placenta, amniotic fluid, fetal membranes, and cord blood from healthy, term pregnancies have been shown to harbour microorganisms, suggesting that the presence of bacteria in these tissues is not necessarily indicative of a pathogenic state (Jiménez *et al.*, 2005; Steel *et al.*, 2005; Rautava *et al.*, 2012; Aagaard *et al.*, 2014). Furthermore, the application of culture-dependent and culture-independent methods have demonstrated that meconium is not sterile but contains bacterial communities similar to those detected in amniotic fluid (Jiménez *et al.*, 2008a; Gosalbes *et al.*, 2013; Ardissone *et al.*, 2014). Taken together, these results challenge the assumption of a sterile *in utero* environment and suggest that initial colonization of the GIT may begin before birth.

Potential sources of these pioneering microbes include the vaginal microbiome (Aagaard *et al.*, 2012; Romero *et al.*, 2014), resident bacteria in the uterus (Hemsell *et al.*, 1989; Cowling *et al.*, 1992; Møller *et al.*, 1995), and the maternal digestive track including the oral cavity (Aagaard *et al.*, 2014). The vagina harbours an abundant, low diversity microbiome dominated by *Lactobacillus* spp. and its proximity to the fetus makes it a possible candidate for fetal colonization (Aagaard *et al.*, 2012). However, bacterial taxa identified in the fetal environment are diverse and include taxa associated with the oral and GIT microbiomes such as *Fusobacterium* spp. and *Bacteroides* spp., suggesting the vagina may not be the primary source of inoculum (Rautava *et al.*, 2012; Aagaard *et al.*, 2014). Recent studies have demonstrated that placentas from healthy, term pregnancies harbour a low abundance but diverse microbiome most akin to the maternal oral microbiome, suggesting that bacteria translocated from the oral cavity may colonize the placenta and serve as an initial source for fetal exposure (Aagaard *et al.* 2014; Zheng *et al.*, 2015). Oral bacteria may enter the blood stream via minor abrasions in the oral mucosa (Lockhart *et al.*, 2008), and preterm birth is associated with both periodontal disease and intrauterine infection, suggesting hematogenous dissemination of bacteria from the oral cavity may be involved in both physiological and pathological uterine bacterial colonization (Goepfert *et al.*, 2004; Klein and Gibbs, 2005; Schwendicke *et al.*, 2015). However, these routes of colonization remain largely speculative, and further detailed studies are needed to characterize the impact of the prenatal maternal microbiome on offspring colonization.

Taking into account that development of the GIT microbiome may begin before birth, we must explore the factors and exposures that influence this process. It is well established that diet modulates the GIT microbiome in adults (David *et al*., 2014), and recent studies have revealed that maternal diet likely influences the offspring's microbiome as well. In a nonhuman primate study, a high fat maternal diet (36% fat) during gestation and lactation was associated with a persistent microbial dysbiosis in offspring even after they were weaned onto an isocaloric control diet (13% fat) (Ma *et al*., 2014). While this study failed to separate the effects of maternal diet during gestation and lactation, another study demonstrated that excess gestational weight gain, but not obesity, is associated with changes in the placental microbiome, suggesting maternal diet during gestation may modulate colonization *in utero* (Antony *et al*., 2015). In addition to maternal diet, antenatal infection (Aagaard *et al*., 2014) and maternal stress during pregnancy (Bailey *et al*., 2004) are associated with changes in the placental microbiome. Moreover, pregestational diabetes (Hu *et al*., 2013) and prenatal probiotic supplementation (Lahtinen *et al*., 2009) correlate with significant changes in the offspring's microbiome. Together, this evidence suggests that the prenatal period plays a significant role in shaping the offspring microbiome.

The microbiome at birth: perinatal influences

Compared to the adult microbiome, the GIT microbiome at birth has significantly lower diversity and higher variability among individuals (Fig. 1A) (Dominguez-Bello *et al*., 2010; Yatsunenko *et al*., 2012). The dominant phyla in the neonatal GIT include Firmicutes, Proteobacteria and Actinobacteria with lower levels of Bacteroidetes, a dominant phylum in the adult GIT microbiome (Fig. 1A) (Gosalbes *et al*., 2013; Ardissone *et al*., 2014; Del Chierico *et al*., 2015). Culture-based studies have reported a predominance of *Bifidobacterium* spp. (phylum Actinobacteria) in the neonatal microbiome (Penders *et al*., 2006). These taxa are known to elicit beneficial effects in their host by breaking down dietary carbohydrates and interacting directly with host metabolism (Davis *et al*., 2011). However, many culture-independent studies have reported relatively low abundances of Actinobacteria in the neonatal microbiome (Fig. 1A). This inconsistency may be explained by poor PCR amplification of *Bifidobacteria* spp. due to variation in the conserved regions of the 16S rRNA gene. In general, this circumstance highlights the need to consider extraction and PCR bias when evaluating results from studies employing molecular methods (Milani *et al*., 2014).

Gestational age at delivery as well as mode of delivery influence the neonatal microbiome at birth (Dominguez-Bello *et al*., 2010; Ardissone *et al*., 2014). Compared to infants delivered at term, the neonatal microbiome of preterm infants exhibits overall lower diversity and lower abundance of *Lactobacillus* spp., *Bacteroides* spp. and *Bifidobacterium* spp., with some differences persisting until 90 days postpartum (Arboleya *et al*., 2011, 2012, 2015). A study of a large cohort of preterm infants demonstrated that gestational age at birth appears to influence the pace, but not necessarily the progression of bacterial colonization, and that gestational age has a larger influence on the progression of colonization compared to other exogenous factors including antibiotic use, diet, and mode of delivery (La Rosa *et al*., 2014). This association may be explained by a microbiota-driven process involved in the pathogenesis of preterm birth, an altered neonatal environment of the preterm infant or possibly an interruption in fetal colonization *in utero*. While much has yet to be learnt about the importance of these factors, recent evidence suggests that the GIT microbiome of preterm infants is largely shaped by microbiota derived from feeding and intubation as well as the immediate incubator and room environment within neonatal intensive care units rather than by individuals who come into contact with the infants (parents and healthcare providers) (Brooks *et al*., 2014). Regardless of the main factors at play, the microbial perturbations associated with preterm birth are considered to be problematic for the infant, as necrotizing enterocolitis, a severe inflammatory disease of the GIT, is strongly associated with both preterm birth and microbial dysbiosis (Wang *et al*., 2009; Mai *et al*., 2011).

In addition to gestational age, recent studies suggest that mode of delivery may influence the immediate neonatal microbiome at birth, although the longer-term impact remains unclear. In the immediate neonatal period, Cesarean delivery is associated with an overall lower bacterial diversity, a lower abundance of *Bacteroides* spp., *Bifidobacterium* spp. and *Lactobacillus* spp. and a bacterial composition akin to maternal skin, while the meconium microbiome of vaginally delivered infants is more similar to the mother's vaginal microbiome (Grönlund *et al*., 1999; Penders *et al*., 2006; Biasucci *et al*., 2008, 2010; Dominguez-Bello *et al*., 2010; Jakobsson *et al*., 2014). However, many of these studies were limited in sample size and failed to stratify subjects based on indication for Cesarean delivery (e.g. unlabored repeat Cesarean versus labored with fetal macrosomia). Thus, differences attributed to mode of delivery may be due to the underlying pathology or fetal physiology that necessitates initial Cesarean delivery. A recent study has found that, in an attempt to recapitulate inoculation during passage through the birth canal, exposure of Cesarean-delivered infants to maternal vaginal fluids at birth results in a relatively minimal effect on the bacterial community profile of anal swab samples, reflecting the partial restoration of a few bacterial taxa (*Lactobacillus* spp. and *Bacteroides* spp.) when compared to levels found in samples from vaginally delivered

infants (Dominguez-Bello *et al*., 2016). At present, it is difficult to definitively determine the significance of these findings with respect to the GIT microbiome, as for example the presence of these taxa may be due to direct inoculation of vaginal bacteria to the perianal region during the inoculation procedure rather than actual changes in the GIT microbiome. Overall, further studies employing larger sample sizes, longer sampling duration and stratification by delivery indication are necessary to determine if failure to pass through the birth canal has a significant, lasting impact on infant microbial development.

The infant microbiome: development during the first year of life

The first year of life represents a significant period of fluctuation and maturation of the GIT microbiome (Fig. 1A). Taxonomic diversity is relatively low at birth but increases over time as the infant is colonized with bacteria acquired from breast milk and the environment (Fig. 1A) (Schanche *et al*., 2015; Thompson *et al*., 2015).

Diet is a significant driver of the developing infant microbiome as it adapts to the changing availability of nutrients (Thompson *et al*., 2015). Early in infancy, the GIT microbiome is enriched in genes involved in digestion of oligosaccharides found in breast milk, while later in infancy, due to the introduction of solid foods, the metagenome is enriched in genes involved in the digestion of polysaccharides (contributed for example to the microbiome by *Bacteroides* spp. (Ravcheev *et al*., 2013)) and vitamin biosynthesis (Koenig *et al*., 2011; Bäckhed *et al*., 2015). Furthermore, the mode of feeding significantly influences microbial composition in the infant GIT (Thompson *et al*., 2015). Breast-fed infants show an increase in the relative abundance of Actinobacteria and a decrease in Firmicutes and Proteobacteria, while formula-fed infants exhibit enrichments in putative pathogens, including *Escherichia coli* and *Clostridium difficile* (Penders *et al*., 2006; Fallani *et al*., 2010; Azad *et al*., 2013; Lee *et al*., 2015). Breast milk contains multiple components that have the potential to impact the composition of the infant GIT microbiome, including immunoglobulins (Rogier *et al*., 2014), prebiotic oligosaccharides (which favor the growth of *Bifidobacterium* spp.) (Hardy *et al*., 2013), and diverse breast milk microbiota that continually seed the infant GIT (Hunt *et al*., 2011). Intriguingly, the breast milk microbiome includes bacteria possibly derived from the maternal GIT via enteromammary trafficking, a proposed pathway by which enteric bacteria are engulfed by leukocytes and delivered to the mammary glands via systemic circulation (Stagg *et al*., 2003; Perez *et al*., 2007; LaTuga *et al*., 2014). In support of this potential pathway are results showing that oral probiotics given to lactating mothers are detectable in breast milk and identical bacterial strains are shared between the maternal GIT, breast milk and the infant GIT (Jiménez *et al*., 2008a,b; Jost *et al*., 2014). Thus, enteromammary trafficking may represent a mechanism for vertical transmission of the GIT microbiome. Given that diet significantly affects the adult GIT microbiome (David *et al*., 2014), the trafficking from maternal to infant GIT may explain the observation that high fat maternal diet is associated with persistent microbial dysbiosis in nursing offspring (Ma *et al*., 2014). However, further studies are clearly needed to elucidate the impact of the maternal GIT microbiota on the developing microbiome of nursing infants.

In addition to infant diet, environmental and pharmacological exposures are also associated with differences in the developing infant microbiome. These factors include antibiotic exposure (Penders *et al*., 2006; Mangin *et al*., 2010), number of siblings (Penders *et al*., 2006), exposure to pets (Nermes *et al*., 2015), daycare attendance (Thompson *et al*., 2015) and geography (Fallani *et al*., 2010). Finally, the interindividual variation within the GIT microbiome is much larger in infants compared to adults (Yatsunenko *et al*., 2012) suggesting that early successional patterns are not uniform, but nevertheless reach a relatively similar endpoint in the adult microbiome (Yatsunenko *et al*., 2012).

The GIT microbiome in childhood

Based on initial culture-based surveys, it was thought that the human GIT microbiome reaches a mature state of colonization between the ages of 1–4 years (Ellis-Pegler *et al*., 1975). However, more recent molecular studies have uncovered key differences in the GIT microbiome of children and adults (Hopkins *et al*., 2001; Cheng *et al*., 2015; Hollister *et al*., 2015). A study in the United States revealed that compared to the adult GIT microbiome, there is an overall clear enrichment in Firmicutes (Cheng *et al*., 2015; Hollister *et al*., 2015), Proteobacteria (Saulnier *et al*., 2011) and Actinobacteria (Cheng *et al*., 2015; Hollister *et al*., 2015) and a decrease in Bacteroidetes (Saulnier *et al*., 2011) in the GIT microbiome of American children. Furthermore, *Roseburia* spp., *Faecalibacterium* spp., *Ruminococcus* spp., *Alistipes* spp., *Bacteroides vulgatus* and *Bacteroides xylanisolvens* were all found to be enriched in preadolescent American children when compared to adult counterparts (Hollister *et al*., 2015). *Roseburia* spp., *Faecalibacterium* spp. and *Ruminococcus* spp. are butyrate-producing bacteria which are associated with a healthy GIT microbiome (Khan *et al*., 2012; Neyrink *et al*., 2012; Zhang *et al*., 2015). On a functional level, the GIT microbiomes of children in the United States are enriched in functions which may support ongoing development, e.g. vitamin B_{12} (Hollister *et al*., 2015) when compared to American adults. The functional repertoires appear to shift over time and culminate in the adult GIT microbiome. Moreover, as we age the

GIT microbiome is enriched in more traits associated with inflammation and metabolic dysfunction (Hollister *et al*., 2015).

As with other stages of life, there also appear to be clear geographical differences with respect to the childhood microbiome. In particular, the GIT microbiome of Italian children is mainly comprised of Firmicutes, Bacteroidetes and a smaller proportion of Actinobacteria (Fig. 1B). In contrast, children residing in rural villages in Burkina Faso exhibit a significant enrichment of Bacteroidetes and depletion of Firmicutes and an enrichment in bacterial genes capable of degrading cellulose and xylan (De Filippo *et al*., 2010). To date, the functional characteristics of the microbiome of children in all other geographical regions remain to be explored in detail. Overall, the GIT microbiome in childhood, while more established than the infant microbiome, is not completely mature and, therefore, represents a dynamic community which likely influences health outcomes later on in life.

The GIT microbiome in adolescence

Similar to the child microbiome, it was assumed that the microbiomes of adolescents are no different than those of adults. However, a single molecular study has identified differences in the adolescent GIT microbiome, including a higher abundance of *Clostridium* spp. and *Bifidobacteria* spp. compared to the adult microbiome (Hopkins *et al*., 2001). Research on the healthy GIT microbiome in adolescence barely exists and future studies are clearly needed in this area. In particular, as fluctuating hormone levels are a hallmark of adolescence, changes in the GIT microbiome likely also occur during this important transitional period similar to other life events associated with major hormonal changes, e.g. pregnancy (Koren *et al*., 2012).

The GIT microbiome in adulthood

The majority of microbiome studies to date have focused on the adult GIT microbiome, which comprises mainly Firmicutes, Bacteroidetes and Proteobacteria (Fig. 1B) (Huttenhower *et al*., 2012). The proportion of each phylum varies according to geographical location (Schnorr *et al*., 2014). For example, a few studies have shown that Firmicutes are enriched in adults in nonindustrialized countries (Schnorr *et al*., 2014; Martinez *et al*., 2015), whereas adults in westernized societies appear to exhibit a higher Bacteroidetes to Firmicutes ratio (Huttenhower *et al*., 2012; Zhu *et al*., 2015). However, this trend is not reflected in two separate studies in which enrichments in Firmicutes were observed for GIT microbiomes from European countries (Mueller *et al*., 2006; Arumugam *et al*., 2011). A higher ratio of Firmicutes to Bacteroidetes has been mostly attributed to energy harvest and body weight gain (Turnbaugh *et al*., 2006). Additional future work is required to clarify the relative levels of Firmicutes and Bacteroidetes and their associated functionalities in healthy human populations across different geographies and their potential impact on health and obesity.

Overall the adult GIT microbiome remains relatively stable through adulthood, except following perturbations such as infections, antibiotic treatment or drastic dietary interventions (David *et al*., 2014). Even though the GIT microbiome recovers to its initial state relatively quickly (Wu *et al*., 2011; David *et al*., 2014), these perturbations subtly alter the GIT microbiome composition over time and are likely strong drivers behind the extensive strain-level interindividual differences which are apparent in healthy adults (Zhu *et al*., 2015). Taken together, this suggests that the human hologenome [the collective of human and microbial genomes (Moran and Sloan, 2015)] is very much individual-specific.

The GIT microbiome in old age

Studies from Europe suggest that the GIT microbiome in the elderly is distinct from adults (Fig. 1B) (Mariat *et al*., 2009). The GIT microbiome is thought to influence the overall health of the elderly, as changes in its composition have been associated with declines in health (Claesson *et al*., 2012). Overall, the GIT microbiome of the elderly exhibits a higher Firmicutes to Bacteroidetes ratio when compared to adults (Mariat *et al*., 2009) with a concomitant reduction in protective commensal bacteria such as *Bifidobacteria* and *Bacteroides* (Mariat *et al*., 2009). A reduction in *Bacteroides* spp., *Prevotella* spp. and *Faecalibacterium prausnitzii* (Mariat *et al*., 2009; Cho and Blaser, 2012), and an increase in *Enterobacteriaceae*, has been associated with on overall decrease in the quality of life in old age (Van Tongeren *et al*., 2005). Factors which play a greater role in old age and which might lead to the observed changes in GIT microbiota include an overall increased use of medication, dietary deficiency, as well as changing hormonal levels (Voreades *et al*., 2014). In order to study the dynamics of the healthy microbiome as we age, further in-depth studies, including a focus on the identification of specific microbiome-conferred functionalities in different geographical regions are necessary. Furthermore, as impaired dentition accompanies old age, it may be worthwhile to explicitly investigate the oral microbiome as this is likely a contributor to changes in GIT microbiota (Claesson *et al*., 2012) and consequently health status.

Age-independent influences on the GIT microbiome and its modulation

Apart from age-associated changes in the GIT microbiota of healthy individuals, other factors including geographical location, social context, gender, host genetics, diet and

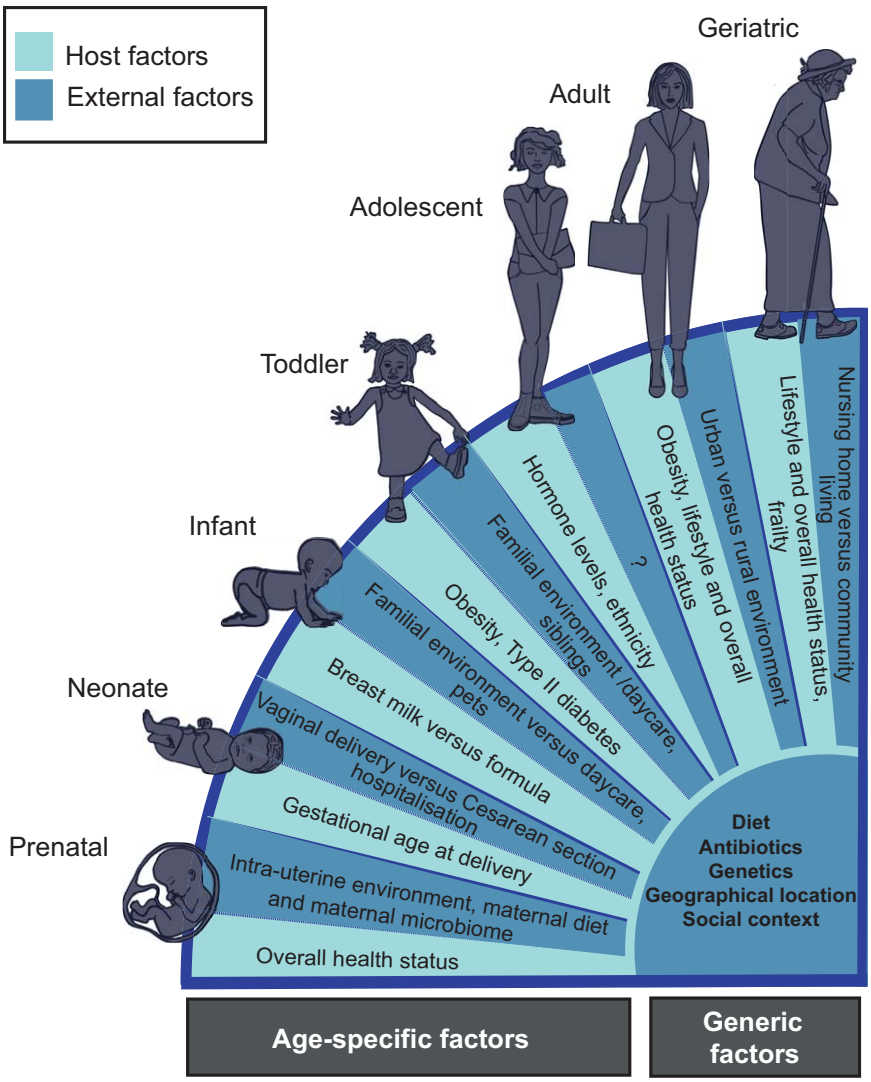

Fig. 2. Factors which influence the gastrointestinal tract microbiome according to different life stages.

antibiotic use may also greatly impact the composition of the GIT microbiome (Fig. 2) (Dethlefsen *et al.*, 2008; Walker *et al.*, 2011). In particular, apparent geographical differences, for example between rural and urban areas (Tyakht *et al.*, 2013), may be attributable to variations in dietary habits or other environmental influences (Fig. 2) (De Filippo *et al.*, 2010; Rampelli *et al.,* 2015; Nakayama *et al.*, 2015). Apart from these factors, the differences observed between school children attending private schools compared to children living in impoverished conditions may be the result of several social factors which are presently difficult to deconvolute (De Mello *et al.*, 2009). These factors likely also contribute to the described association between taxonomic structures and level of education (Ding and Schloss, 2014). Furthermore, elderly individuals that live in a community setting have higher proportions of Firmicutes to Bacteroidetes in comparison to long-stay residential individuals (Fig. 2) (Claesson *et al.*, 2012). Data from the Home Microbiome Project has found that the human microbiome fingerprint is very similar between individuals that share the same home (Lax *et al.*, 2014) which in turn suggests an overall high degree of nestedness for the GIT microbiome which likely further reinforces social effects. These results as well as those from other studies (Yatsunenko *et al.*, 2012) suggest that the overall environmental setting is a strong factor in shaping the human GIT microbiome and, in more general terms, the microbiomes of other body habitats.

Apart from environmental factors, host genetics have also been found to shape GIT microbiome composition (Goodrich *et al.*, 2014; Blekhman *et al.*, 2015). More specifically, many microbial taxa whose abundances appear to be affected by host genetics have been identified (Goodrich *et al.*, 2014). Interestingly, the most heritable taxon identified, the family Christensenellaceae, was found to be enriched in individuals with low body mass index. When

transplanted into germ-free mice, a Christensenellaceae amendment reduced weight gain significantly and overall altered the microbiome of recipient mice (Goodrich *et al*., 2014). Strikingly, the heritability of Christensenellaceae did not appear to be driven by diet and was, therefore, mainly due to host genetic factors (Goodrich *et al*., 2014). Furthermore, gender-specific differences in GIT microbiome composition have been observed, which may be explained by combinations of host genetics and social context (Markle *et al*., 2013; Ding and Schloss, 2014).

Although diet appears not to be the main driver influencing microbiome composition, certain major taxa, e.g. the Bacteroidetes, have been found to respond very rapidly to dietary changes (David *et al*., 2014). In particular, Western dietary habits have been found to significantly affect the GIT microbiome which in turn has been linked to metabolic diseases (Fukuda and Ohno, 2014; Hur and Lee, 2015). The Western diet consists largely of processed foods and is high in simple carbohydrates, animal protein, fat and low in dietary fibre (De Filippo *et al*., 2010). In contrast, high fibre diets are typically composed of fresh vegetables, fruits and whole grains (Fung *et al*., 2001). Fibre-rich diets can have short term-impacts on the GIT microbiome (David *et al*., 2014), but more importantly long-term fibre intake has been linked to microbiome-mediated beneficial, systemic effects (Kuo, 2013). Interesting in this context are *Prevotella* spp. and *Xylanibacter* spp., two genera capable of degrading nondigestible fibres, which are enriched in children from Burkina Faso and completely absent in European children (De Filippo *et al*., 2010). Fibre-degrading bacteria ferment dietary fibre to SCFAs (Flint *et al*., 2012), which provide an important source of energy for host cells (Bäckhed *et al*., 2004; Turnbaugh *et al*., 2006) as well as being involved in the maintenance of the epithelial barrier (Kelly *et al*., 2015) and modulation of the immune system (Furusawa *et al*., 2013). The two latter processes are particularly pertinent in the context of allergic diseases, which show an increasing incidence world-wide, particularly in Western countries (Azad *et al*., 2013). More specifically, increased circulating levels of fibre-derived SCFAs have been found to protect against inflammation in the lung of a mouse model, whereas a low-fibre diet decreased levels of SCFAs and increased allergic airway disease (Trompette *et al*., 2014). Additionally, even though the results are not conclusive, it seems that there is potential that the co-administration of nondigestible fibre during antibiotic treatment has promising effects on the GIT microbiome such as prevention or alleviation of antibiotic-associated diarrhea (Moore *et al*., 2015).

Epidemiological data suggests that the increasing prevalence of antibiotic usage and general cleanliness in Western countries ('the hygiene hypothesis') negatively affects the GIT microbiome and that these contribute to the increasing incidence in allergic diseases in these countries (Azad *et al*., 2013). The administration of antibiotics directly perturbs the GIT microbiome leading to decreased bacterial diversity and richness (Dethlefsen *et al*., 2008; Claesson *et al*., 2011). Even though the GIT microbiome is restored after approximately one month (Claesson *et al*., 2011), some taxa are permanently lost (Dethlefsen *et al*., 2008). However, these and other studies (Penders *et al*., 2006; Mangin *et al*., 2010) have only investigated microbial diversity before and after interventions and have not examined transient and subsequent microbial metabolic profiles nor the molecular repercussions over longer durations. Even though antibiotic exposure might only have relatively short-term effects on the microbiota, it might activate long-term changes in gene expression that could affect the immune system or metabolism which in turn might culminate in chronic diseases (Nobel *et al*., 2015). Furthermore, facultative pathogens may occupy larger niches postadministration which may lead to sustained pro-inflammatory states (Gagliani *et al*., 2014). Clearly, detailed longitudinal studies aimed at understanding the long-term impact of antibiotic treatment on microbiome-mediated health are needed to fully understand the resilience of the GIT microbiome in relation to antibiotic exposure and to identify possible supportive treatment strategies.

A well-established instance where persistent antibiotic use leads to a major disruption of the GIT microbiota balance with potentially life-threatening consequences is that of recurrent *Clostridium difficile* infection, which is particularly prevalent in elderly individuals. In terms of treatment, fecal microbiota transplantation (FMT) has recently proven to be very efficacious as it results in the reestablishment of a more diverse, 'healthy' microbiome (Kelly *et al*., 2014; Dutta *et al*., 2014; Cohen *et al*., 2015). Apart from FMT, other more classical means for modulating the GIT microbiome include the administration of defined pre-, pro- or syn-biotic formulations (Hardy *et al*., 2013). Prebiotics comprise nondigestible food components that promote the growth of certain beneficial bacteria, e.g. SCFA-producing bacteria, in the GIT. Examples include dietary supplementation with nondigestible fibre which promotes the growth of Actinobacteria (Gerritsen *et al*., 2011; Johnson and Versalovic, 2012; Simeoni *et al*., 2015). Actinobacteria have been shown to be associated with health benefits and to protect against pathogens by modulating the host's immune responses (Ventura *et al*., 2007). Probiotics are living bacteria that can provide health benefits to the host when administered in adequate amounts (Rijkers *et al*., 2011) as they may potentially restore a disturbed GIT microbiome (Sullivan and Nord, 2002) and activate anti-inflammatory pathways (Eloe-Fadrosh *et al*., 2015). Certain probiotic species produce SCFAs and may secrete other molecules, which, *inter alia*, may stimulate the growth of other beneficial bacteria (Eloe-Fadrosh *et al*., 2015) and thereby prevent the expansion of pathogenic

bacteria (Collado *et al.*, 2007). Synbiotics, which involve a combination of probiotics and prebiotics administered together, have been shown to favourably modulate interactions between the GIT microbiome and host, and thereby confer overall health benefits (Druart *et al.*, 2014). More specifically, modulation of the microbiome by synbiotic regimes has been demonstrated to be efficacious for the protection against sensitization to food allergens (Stefka *et al.*, 2014), the reduction of inflammation in the GIT (Furrie *et al.*, 2005) and a corresponding reduction in colorectal cancer risk (Ohashi *et al.*, 2002; Rafter *et al.*, 2007; Liu *et al.*, 2011). Beyond the use of undefined modulatory regimes, i.e. FMT, targeted age-group specific synbiotic regimes may be further developed (Ley *et al.*, 2006; De Filippo *et al.*, 2010) to prevent or/and treat imbalanced GIT microbiota over the course of human life.

Attributes of baseline 'healthy' microbiomes

Even though the GIT microbiome in healthy individuals is considered to be relatively stable (Manichanh *et al.*, 2008; Ding and Schloss, 2014), the identification of features characteristic of a 'healthy' microbiome is a challenging task. Indeed, desirable microbial community structures associated with human health may be different for different individuals according to their age, environment, genetics, diet, etc., and may be more dependent on microbial gene carriage patterns than taxonomic composition (Huttenhower *et al.*, 2012; Zhu *et al.*, 2015). In contrast, given the relative stability of functions encoded in GIT metagenomes (Huttenhower *et al.*, 2012), it is likely that a core of microbiome-conferred functionalities exists. The characteristics of such a 'functional core' should be explored and its specific attributes in relation to human health should be investigated.

Many studies have identified microbial community structures associated with specific diseases and correspondingly, it is tempting to associate 'health' with the features identified in the healthy control cohorts of these studies. However, assigning causality to features associated with health and disease is a difficult problem and often requires use of model systems, which at present do not accurately reflect human physiology (Fritz *et al.*, 2013). Additionally, careful consideration of exclusion criteria should be taken before these features are considered to be reflective of a 'healthy' microbiome, as many studies may identify 'healthy' features which may be associated with other factors not under investigation. For example, many studies have identified a relatively low Firmicutes to Bacteroidetes ratio to be associated with health, as elevated ratios have been linked to metabolic diseases (Ley *et al.*, 2006; Mariat *et al.*, 2009). However, due to considerable variation in the observed Bacteroidetes to Firmicutes ratios in healthy subjects, which may in turn be due to several factors (Ley *et al.*, 2006; Mariat *et al.*, 2009), this ratio is likely not be a generalizable indicator of human health (Hollister *et al.*, 2015).

Large-scale studies of healthy individuals screened with extensive exclusion criteria such as the Human Microbiome Project and MetaHIT, have allowed us to begin to define what is characteristic of healthy individuals, and this should form the framework for defining features of 'healthy' microbiomes in the future. In addition to identifying microbial community structures associated with human health, studies should also focus on identifying specific functionalities and the corresponding bacterial taxa that confer these functions to the individual. For example, some *Bacteroides* spp. are known to produce specific beneficial products such as polysaccharide A (Johnson *et al.*, 2015) and SCFAs (Walker *et al.*, 2005; Ley *et al.*, 2006). Although 16S rRNA gene-based analyses have a role to play for the *ab initio* characterization of community structures, future studies should involve the systematic generation of metagenomic and functional omic [metatranscriptomic, metaproteomic, (meta-)metabolomics] data for resolving the functional complements of microbiomes. Ultimately, the functionalities encoded and conferred by the microbiota are key to ensuring health. Finally, the establishment of a repertoire of beneficial bacterial strains is necessary as their administration as probiotics potentially in conjunction with prebiotic formulations represents one of the most promising avenues of microbiome-related therapeutics.

Characterization efforts aimed at defining 'healthy' microbiota compositions must be expanded beyond the lower GIT microbiome, and links between different body sites must be systematically investigated. For example, the oral cavity is a main gateway for microorganisms to enter and interact with the human body and the community types of the oral and lower GIT microbiomes are predictive of each other (Ding and Schloss, 2014). Additionally, the oral microbiome has been associated with oral infectious diseases as well as systemic diseases (Ley *et al.*, 2006; Seymour *et al.*, 2007). Consequently, the oral microbiome might be a key factor for understanding human health over a human lifetime. Furthermore, it is also likely that alterations to the microbiota in other body habitat, e.g. the skin (Weyrich *et al.*, 2015), may trigger systemic effects not least via immune system responses. Clearly, microbial communities at different body sites are involved in different aspects of human health, and the systematic characterization of many body sites is therefore imperative for our overall understanding of microbiota-mediated health in the future.

Concluding remarks and perspectives

Given the complexity and diversity of microbial community structures in healthy individuals (Huttenhower *et al.*, 2012), it may be at present difficult to define attributes characteristic of baseline 'healthy' microbiomes. In spite of the

relatively conserved, characteristic features of body site-specific microbiota (Kurokawa *et al*., 2007; Huttenhower *et al*., 2012; Hollister *et al*., 2015), it remains unclear which factors influence microbial community assembly and their relative importance. Key factors which likely influence microbiota compositions include age (Palmer *et al*., 2007; Mariat *et al*., 2009; Koenig *et al*., 2011), geographical location (Yatsunenko *et al*., 2012), host genetics (Gagliani *et al*., 2014; Blekhman *et al*., 2015), social context (De Mello *et al*., 2009; Koenig *et al*., 2011; Nakayama *et al*., 2015), and diet (David *et al*., 2014) (Fig. 2). Therefore, in order to understand how the microbiome plays a role in maintaining human health, studies aimed at identifying measures that sustain beneficial microbiome compositions over the course of a human lifetime along with high-resolution longitudinal data in different geographic locales is essential. Such data would need to include information on highlighted factors influencing microbiome composition as well as provide a sampling from different body sites of individuals of different ages, ethnicities, socio-economic backgrounds and geographies. Furthermore, such efforts should go beyond mere 16S rRNA gene-based taxonomic surveys and include systematically generated metagenomic and functional omic data. Given the microbiome's contribution of key functionalities to host physiology, the inclusion of functional information will likely greatly enhance the linking of microbial contributions to overall health status. Additionally, it is essential that all data is stored in a web-accessible comprehensive database to allow comparisons of body-site specific microbiome characteristics across studies. Therefore, in our opinion, a standardized, concerted international effort is needed to resolve and define the healthy human microbiome.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Relative abundance of bacterial phyla in stool samples obtained in different geographical locations worldwide (average %).