The production of a variety of neuroactive signaling molecules by bacterial components of the microbiome provides additional evidence that the gut microbiome may generate signals with remote effects in different organ systems, including the central nervous system

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The Human Microbiome and Probiotics: Implications for Pediatrics by James Versalovic

Key insights

Knowledge of the composition and function of the human microbiome at multiple body sites including the gut, skin and airways contributes to our understanding of the mechanisms of probiosis. In turn, an enhanced understanding of the effects of probiotics on the microbiome should facilitate selection of optimal probiotic strains for the treatment of specific diseases.

Current knowledge

The human microbiome is composed of bacteria, viruses (including bacteriophages), fungi, archaea and protozoa. Each body site has its own distinct microbiome, with a unique microbial composition that presumably reflects the differences in tissue structure and function. For decades, it has been widely accepted that the ingestion of probiotic strains has beneficial effects. The work of the Human Microbiome Project has provided a wealth of data on the taxonomic profiles of over 5,000 bacterial strains from 18 different body sites. A major challenge is to quantify the relative abundance of the different strains in health and disease, before we can harness the full potential of probiotics.

Practical implications

Knowledge of the intestinal microbiome provides an opportunity for understanding the roles of the microbial populations in other parts of the body. The compositional differences between the gut microbiomes of infants who develop necrotizing enterocolitis (NEC) versus healthy infants underscore the need to tip the balance in favor of beneficial strains. Recent studies have demonstrated that probiotics or symbiotics can have a direct impact on gut microbial composition and profoundly affect the clinical outcomes for infants with NEC. The successful applica-

Each body site has a specific microbiome. This coordinates plot displays variation in terms of bacterial composition at different body sites in humans; depicted here are the oral (darkest grey), gastrointestinal (light grey), vaginal (dark grey), nasal (black) and skin (grey) microbiomes. For further details, please refer to the article. Adapted from Huttenhower et al. [Nature 2012;486:207–214].

tion of probiotics for NEC has been extended to the treatment of other intestinal disorders and provides a paradigm for the therapy of other conditions such as asthma.

Recommended reading

McNulty NP, Yatsunenkov T, Hsiao A, Faith JJ, Muegge BD, Goodman AL, Henrissat B, Oozeer R, Cools-Portier S, Gobert G, Chervaux C, Knights D, Lozupone CA, Knight R, Duncan AE, Bain JR, Muehlbauer MJ, Newgard CB, Heath AC, Gordon JI: The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. *Sci Transl Med* 2011;3:106ra.

The Human Microbiome and Probiotics: Implications for Pediatrics

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Key Messages

- The composition and function of the microbiome can be changed by probiotics.
- The composition of the human microbiome at each body site is distinct, and different probiotics are likely needed for diseases at different body sites.
- Microbial deficiencies may be treated by probiotics as a strategy for microbial supplementation and promotion of microbial diversity.
- Probiotics may change the ability of the microbiome to produce nutrients and bioactive compounds by *de novo* biosynthesis or by luminal conversion.

Key Words

Human microbiome · Probiotics · Bacteria · Antibiotics · Microbes · Metagenomics · Beneficial microbes · Dysbiosis

Abstract

Steady advances in our knowledge of the composition and function of the human microbiome at multiple body sites including the gut, skin and airways will likely contribute to our understanding of mechanisms of probiotic action by beneficial microbes. Microbe:microbe and microbe:human interactions are important considerations as we select probiotics for pediatric patients in the future. Although our

knowledge about the composition of the microbiome is progressing rapidly, many gaps exist about the functional capacity and metabolic machinery of the human microbiome. Based on a limited amount of data, probiotics appear capable of altering the composition and function of the microbiome. Probiotics may be part of dietary strategies that combine ways to enhance microbiome function with nutrients that may be converted to active compounds promoting human health. Probiotics have yielded beneficial effects in numerous studies in the context of different diseases in pediatric gastroenterology. These disease states include necrotizing enterocolitis, antibiotic-associated diarrhea and colitis, acute gastroenteritis and irritable bowel syndrome. In the skin and airways, it is unclear if probiotics can affect the function of the microbiome to reduce the impact of diseases such as asthma and atopic dermatitis. An enhanced understanding of the effects of probiotics on the microbiome should facilitate selection of optimal probiotic strains for specific diseases in the future.

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Pediatrics, the Microbiome and Probiotics

The practice of pediatrics, as in other medical specialties, has viewed microorganisms with a defensive posture and considered each bacterium, fungus or virus as a potential infectious agent. The prevailing 'infectious diseases/antimicrobial' strategic world view in medicine be-

came a predominant view since the first 3 decades of the twentieth century. As infectious agents and infectious diseases were being characterized a century ago, the beginning of antimicrobial agent discovery and consideration of antibiotics as novel treatments began to take shape. In fact, phage therapy as an antimicrobial strategy in medicine was being considered during the second decade of the 1900s (reviewed by Pirisi [1] and Keen [2]), and arsphenamine (Salvarsan) and its less toxic derivative (Neosalvarsan), discovered by Ehrlich and Hata in 1910, were being applied to treat different infections. In 1928 (reprinted by Fleming [3]), Sir Alexander Fleming's discoveries led to the identification of antibacterial compounds produced by specific fungi and laid the foundation for the antimicrobial era. This prevailing world view in medicine dominated the landscape of pediatrics until the first decade of this century, when the attitudes towards commensal and beneficial microbes began to change profoundly.

Beneficial microbes and more specifically probiotics were described initially by Nobel laureate Elie Metchnikoff [4] in 1907/1908, when he described the potential benefits of consumption of large quantities of microbes to improve longevity and human health. Unfortunately, this viewpoint was effectively subordinated to the view that microbes must be considered as potentially infectious agents, and most attention in the medical profession including pediatrics turned to vaccine development and antibiotic production. The term 'probiotic' was first credited to Lilly and Stillwell [5] who proposed this term in the context of microbes producing substances that promoted the growth of other microorganisms. Parker [6] was the first to use the term 'probiotic' to describe microorganisms (and substances) that have beneficial effects on a host animal. Outside of the dairy and fermented food industry, the probiotic concept remained largely dormant until the late 1980s. The British Professor R. Fuller [7] described the modern probiotic concept in a landmark review published in 1989 and proposed the importance of microbial viability to probiotic function. A formal definition of probiotics was formulated in 2001 by the advisory body of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), and this definition has been widely utilized during the past 12 years. This definition states that probiotics are 'live microorganisms which when adminis-

tered in adequate amounts confer a health benefit on the host' [8].

The emergence of investigations concerning the nature and mechanisms of probiosis during the 1990s and the rapid coalescence of the human microbiome research community globally since 2005 [9] provided the foundation for the current era in metagenomics (the genomic analysis of microorganisms by direct extraction and cloning of DNA from an assemblage of microorganisms). Human microbiology includes canonical and opportunistic

infectious agents, but this field has rapidly expanded to embrace the many commensal and beneficial microbes that may contribute to human health and disease prevention. The specialty of pediatrics has been swept into this new era of human microbiology and

medicine as a result of numerous publications describing the composition of the microbiome in children and differences in the microbiome associated with diseases of childhood [10]. Alterations in microbial composition associated with human diseases have been described as examples of dysbiosis. Dysbiosis refers to differences in microbial populations that may reflect an abnormal ecological state contributing to pathology or the excess of pathogenic mechanisms within the human microbiome [see Chan et al. in this issue]. Functional components of the microbiome may be studied by determination of DNA sequences or genes present in the microbiome, but this information only reveals the metabolic capacity. RNA sequencing and metabolomics studies are necessary to determine which microbial genes are expressed and which metabolites may affect disease susceptibilities in children.

The human microbiome is composed of bacteria, viruses (including bacteriophages), fungi, archaea and protozoa in declining order. Human-associated bacterial species comprise the vast majority of the human microbiome in terms of microbial DNA content and cell count. In fact, in one recent study, more than 99% of mapped DNA sequencing reads in healthy adults were bacterial sequences [11]. Human-associated bacterial communities are composed of four dominant phyla (Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria) and a number of minority phyla [12]. A recent study of the biogeography of the human microbiome identified 30 phyla in 18 different body sites in neonates and adults [13], and reports of cultured and uncultured bacteria estimates ap-

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Fig. 1. The microbiome differs at each body site. This principal coordinate (PC) plot displays variation in terms of human bacterial composition at different body sites. The oral (darkest grey), gastrointestinal (light grey), vaginal (dark grey), nasal (black) and skin (grey) microbiomes are shown in different shading. Each point in two-dimensional space represents a different healthy human individual and relative distances between the sites indicate relative differences in bacterial composition (adapted from Huttenhower et al. [12]).

proximate numbers exceeding 50 total human-associated bacterial phyla [14, 15]. The composition of the microbiome is distinct at each body site or habitat, each body site has a distinct microbial composition and, presumably, differences in function translate to differences in physiology at each body site (e.g. skin and intestine) (fig. 1).

The relative abundance of probiotic genera and species in the healthy human microbiome is a relevant consideration, as well as whether microbial deficiencies in individual species could be readily corrected by administration of probiotics to children. Alternatively, do probiotics simply enhance the ability of other bacterial genera to proliferate and reduce the numbers of potentially harmful bacteria? Rational probiotic strategies could be developed that take advantage of predictable changes in microbial composition following probiotic therapy. For 2 decades, we had scientific evidence that ingestion of probiotics in human volunteers resulted in persistence of probiotic strains (lactobacilli) 11 days later in the small intestine with corresponding reductions in other bacte-

rial genera [16]. The initial comprehensive summary of the Human Microbiome Project included 242 individuals with greater than 5,000 bacterial taxonomic profiles from 18 body sites in healthy adults [11, 12]. The Firmicutes and Bacteroidetes were the dominant phyla in the gastrointestinal tract, and *Lactobacillus* was a minority Firmicute genus in these individuals. The phylum Actinobacteria includes the genus *Bifidobacterium* which has been underrepresented in human microbiome studies due to technical challenges in DNA amplification/detection methods. Focused efforts to quantify the relative abundance of *Bifidobacterium* have determined that this genus is present as a minority genus in the colon.

Human Gut Microbiome, Diet and Probiotics

Human nutritional components and dietary patterns clearly impact microbial composition and presumably function in the intestine. Children consuming a high-fiber, plant-based diet in western Africa demonstrated a relative abundance of two bacterial genera, *Prevotella* and *Xylanibacter*, that may contribute to digestion of plant components and fiber in the diet. These two genera contain genes and pathways capable of metabolizing cellulose and xylan in the diet, and these genera are rare or absent in children consuming a westernized diet in southern Europe [17]. A more recent study also described major differences in the fecal microbiomes of children in the USA and Bangladesh [18]. Although fundamental long-term differences in the diet clearly seem to affect gut microbial composition, short-term (days) major changes in diet do not have a correspondingly major impact on gastrointestinal microbial composition [19]. It appears that major changes in the diet will require longer time periods (months to years) to profoundly shift the composition of the gut microbiome in human individuals. As health care providers consider probiotic strategies, it is important to also keep in mind that short-term consumption of probiotics does not appear to shift microbial composition, but studies in mouse models suggest that the major impact of probiotics in the short term may pertain to changes in microbial gene expression and metabolite production [20].

In addition to the effects of diet and probiotic consumption on the composition of the gastrointestinal microbiome, prebiotics or symbiotic combinations may also affect gut microbial composition. Gibson et al.'s [21] landmark paper in 1995 provided evidence for the prebiotic concept and the idea that indigestible oligosaccharides would provide a substrate for beneficial microbes in

the intestine. Human milk oligosaccharides (HMOs) have been characterized in recent years as a distinguishing feature from bovine milk, and these milk components appear to promote the proliferation and biological functions of probiotic genera such as *Bifidobacterium* spp. [22]. For several decades, it has been recognized that breast milk-fed infants demonstrated relative resistance to infectious gastroenteritis [23]. The supplementation of probiotics to infant diets yielded protection against diarrhea and rotaviral infection [24]. So, the concepts of prebiotics, probiotics and effects on the resilience and diversity of the microbiome become intertwined, and a broader consideration of combinations of probiotics with nutritional strategies may result in predictable changes to the function of the microbiome. The 'center stage' importance of nutrition early in life and during childhood and adolescent development highlights the potential impact of probiotics on basic aspects of human nutrition and nutrient availability. Twin pairs in eastern Africa were differentially susceptible to the condition of undernutrition known as kwashiorkor, based on differences of the composition of their intestinal microbiomes [25]. Presumably, susceptibilities to the clinical phenotypes of undernutrition are affected by the metabolic capacity of the microbiome and the abilities of gut bacteria to convert foodstuffs into available nutrients for childhood development.

Gut bacteria synthesize and convert a variety of compounds that impact the physiology, immunity and presumably disease susceptibility or resistance of human individuals. Examples of de novo biosynthesis pathways in the gut microbiome and probiotics include the synthesis of B complex vitamins such as vitamin B₁₂ (cobalamin) [26] and vitamin B₁ (thiamine) [27]. Examples of luminal conversion include the conversion of plant lignins to enterolignans, vitamin K₁ (phyloquinone) to vitamin K₂ and analogs (menaquinones) [28], amino acid decarboxylation reactions generating biogenic amines (e.g. histidine/histamine, glutamate/GABA) [29, 30] and carbohydrate/dietary fiber conversion to short chain fatty acids (SCFAs) [31]. With respect to amino acid metabolism, oral administration of probiotic *Bifidobacterium* species can stimulate peripheral blood-derived immune cells to produce greater amounts of the immunosuppressive cytokine interleukin-10 (IL-10) and the immunosuppres-

sive enzyme indoleamine 2,3-dioxygenase (IDO) [32]. IDO facilitates the conversion of the amino acid tryptophan to the metabolite kynurenine, and this metabolic conversion has been associated with virus- and tumor-induced immunosuppression. Kynurenine may suppress inflammation in the context of a proinflammatory micro-

biome or host condition. Conjugated linoleic acids are fatty acid derivatives produced by various probiotic species, generating compounds with anti-inflammatory and anticarcinogenic effects [33]. Probiotic *Lactobacillus plantarum* administration in dairy goats resulted in shifts in gut microbial composition and altera-

tions in milk fat composition including greater amounts of polyunsaturated fatty acids such as linoleic acid [34]. In summary, the human microbiome contains an enormous capacity for converting nutrients and dietary substrates, and this functional capacity can be affected by administration of probiotic strains.

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Microbiome, Probiotics and Pediatric Gastroenterology

Early Microbial Ecology in the Gut

The microbial ecology of the human intestine provides an opportunity to understand the nature of microbial populations in the human body and how these populations influence gene expression patterns and the ultimate functionality of the microbiome. It is unclear whether the fetus is exposed to microbes or their metabolites or DNA, although investigators are actively exploring the relationship of the human microbiome to the in utero environment and mode of delivery [see Luoto et al. in this issue]. During infancy, the composition of the gut microbiome fluctuates rapidly [35] and reaches an adult-like equilibrium by 3 years of age in one study [36] (fig. 2).

Necrotizing Enterocolitis

The development of the intestinal microbiome in early life and its importance has been highlighted in the pre-term neonate at risk for developing necrotizing enterocolitis (NEC) [see Walker in this issue]. The gut microbiome of infants who developed NEC was characterized by compositional differences such as increased abundance of γ -Proteobacteria [37], a common feature of the gut microbiome in disease states. Compositional differences in

Fig. 2. Bacterial diversity increases with age. Intestinal bacterial composition increases steadily during the first 3 years of life. Standard errors of the mean are plotted (adapted with permission from MacMillan Publishers Ltd. from Yatsunen et al. [36]). Black circles = US; grey circles = Amerindians; white circles = Malawians; OTU = operational taxonomic unit (bacterial taxon).

the fecal microbiota preceded the development of NEC. Possibly, probiotics or nutritional approaches could shift microbial composition towards a more disease-resistant gut microbiome. A diet composed entirely of human milk has been effective in reducing the incidence of NEC in premature infants, and donor human milk supplementation has been recommended as one strategy to prevent NEC [38, 39]. In addition to human milk supplementation, several probiotics have yielded success in the prevention of severe NEC and all-cause mortality in premature infants, including very-low-birth-weight infants [40, 41]. Data could not be extrapolated to extremely-low-birth-weight infants. Heterogeneity among the clinical trials in terms of design and probiotic strains prevents any firm recommendation regarding a specific probiotic strain for prevention of NEC [42]. An increased incidence of NEC has been associated with administration of histamine 2 receptor (H2R) antagonists as acid blockers to preterm infants [43, 44]. The histamine signaling pathway may provide an opportunity for targeted interventions of probiotics based on known mechanisms. Probiotics capable of converting the dietary amino acid L-histidine to histamine have been reported [29], and histamine may suppress inflammation by promoting H2R signaling in the intestinal mucosa.

Recurrent Clostridium difficile Infection and Acute Gastroenteritis

Disorders of microbial ecology may be caused by consumption of antimicrobial agents (antibiotics) and che-

motherapy, so that iatrogenic infections may be corrected by probiotics. In the past decade, the incidence of pediatric *Clostridium difficile*-associated disease has steadily increased [45]. Gorbach et al. [46] demonstrated successful treatment of *C. difficile* disease using a single strain of human-derived *Lactobacillus rhamnosus* (LGG). These findings laid the foundation for the generalized acceptance of probiotics in the last decade of the twentieth century. This strain of interest became commonly known as the probiotic LGG and has been applied in numerous pediatric studies [47, 48]. Several studies in children have demonstrated that probiotics may be effective at suppressing antibiotic-associated diarrhea [49, 50], and probiotics may promote restoration of microbial diversity as one mechanism for amelioration of the disease phenotype [51]. Prior evidence showed that the human intestinal microbiome was restricted in terms of bacterial diversity in patients with recurrent *C. difficile* disease [52]. Presumably, a gut microbiome with limited diversity (an example of dysbiosis) creates a permissive environment of recurrent colitis due to *C. difficile*, and probiotics may be useful by promoting increased gut bacterial diversity. The success of fecal microbiota or intestinal microbiome transplantation in *C. difficile*-associated disease [53] provides additional evidence that restoration of sufficient bacterial diversity and functional capacity can effectively treat this disorder of microbial ecology.

The American Academy of Pediatrics (AAP) endorsed the application of probiotics for the prevention of antibiotic-associated diarrhea and the treatment of acute viral gastroenteritis in healthy children [54]. Although data are lacking with respect to changes in the intestinal microbiome in cases of acute bacterial or viral gastroenteritis in humans, probiotics have demonstrated their ability to shorten the course of disease and ameliorate symptoms in several studies spanning 2 decades [49, 55]. The addition of probiotics may stimulate the mucosal immune system and the microbiome's own defense mechanisms, resulting in rapid pathogen clearance and mucosal healing.

Celiac Disease

Although the microbial composition of the small intestine does not appear to differ in patients with celiac disease, differences in the intestinal microbiome were detected in self-collected stool specimens obtained from patients with celiac disease [56]. Corresponding changes in the fecal microbiome and the fecal and urinary metabolome were reported in children with celiac disease compared to healthy controls [56]. Breast milk feeding with

its possible ‘feeder’ effects on beneficial microbes and HLA genotype were found to influence the relative susceptibilities to celiac disease in the PROFICEL study [57]. In a provocative report, the relative timing of gluten introduction in early childhood appeared to impact disease susceptibility, and the disease phenotypes were correlated with changes in the intestinal microbiome and metabolome [58]. An interesting aspect relevant to celiac disease is the presence of gluten-metabolizing bacterial genera such as *Rothia* spp. in the oral microbiome [59]. Gluten-metabolizing microbes in the oral or intestinal microbiomes may reduce the relative susceptibilities of individuals with a genetic predisposition to celiac disease due to HLA gene polymorphisms. Future probiotic strategies may include consideration of probiotics with gluten metabolism genes and the ability of probiotics to enhance the function of such gluten-metabolizing bacteria in the microbiome.

Irritable Bowel Syndrome

Differences in composition or dysbiosis of the gut microbiome in irritable bowel syndrome (IBS) were first reported with comprehensive DNA sequencing and array studies in 2011 [60, 61]. Disease signatures based on differences in bacterial composition were detected and distinguished patients with more frequent abdominal pain and more severe gastrointestinal disease phenotypes. Overlapping features included the enrichment of γ -Proteobacteria in children and adults with IBS, and an association of this group containing known enteric pathogens with increased pain symptoms. Subsequent studies confirmed and extended these findings regarding distinct compositional differences of the intestinal microbiome in IBS [62–64]. In children, a follow-up study described differences in the fecal microbiome such as relative deficiencies of the genera *Bifidobacterium* and *Verrucomicrobium* in children with IBS-diarrheal predominant subtype (IBS-D) [65]. Administration of *Bifidobacterium* spp. in adult patients with IBS reduced symptoms and highlighted the potential benefits of probiotic therapies in IBS with carefully selected probiotic strains [66, 67]. Reduced amounts of *Bifidobacterium* spp. in the intestinal microbiomes of children and adults with IBS point towards a rational basis for supplementation of ‘missing’ or deficient bacteria in disease conditions as a way to prevent or treat disease. The suggested importance of intestinal *Bifi-*

dobacteria may explain the relative success of a *Bifidobacterium*-*Lactobacillus* combination strategy [68] versus a *Lactobacillus*-only probiotics strategy [69] in children with IBS.

A general consensus in the field is that many functional gastrointestinal disorders including IBS can best be understood as disorders of brain-gut interactions [70]. The brain-gut axis represents a bidirectional connection between the digestive and nervous systems with emerging importance to human biology and medicine [70]. Recent

Changes in the composition and function of the mammalian gut microbiome can affect brain systems related to pain and affect regulation.

preclinical evidence suggests that changes in the composition and function of the mammalian gut microbiome can affect brain systems related to pain and affect regulation [71]. In rodents, the lack of gut microbes in germ-free mice [72, 73] and the modulation of gut microbial ecology by probiotics [74] and antibiotics [75] have been associated with changes in affective behavior, pain responses and gene expression in the brain. The production of a variety of neuroactive signaling molecules by bacterial components of the microbiome provides additional evidence that the gut microbiome may generate signals with remote effects in different organ systems, including the central nervous system. Recent preclinical data in rodents suggest that changes in the gut microbiota can be associated with changes in the expression of brain signaling systems and associated emotional behavior [72, 74], and oral administration of probiotics to healthy women demonstrated changes in interoceptive, affective and reward circuits in response to chronic probiotic ingestion [76]. In summary, probiotics may be useful for the prevention or treatment of functional gastrointestinal disorders like IBS by affecting the function of the gut microbiome or by altering brain function and pain perception centrally.

Inflammatory Bowel Disease

Differences in the composition of the intestinal microbiome have been reported in several studies of patients with Crohn’s disease and ulcerative colitis. Such differences include reduced proportions of the bacterial phyla Bacteroidetes and Firmicutes, relative deficiencies of the genus *Faecalibacterium* in ileal Crohn’s disease and expansion of the phylum Proteobacteria in patients with inflammatory bowel disease (IBD) [77–79]. Early successes with probiotics in the context of acute and chronic pouchitis [80, 81] have been followed with mixed results and

disappointments in clinical trials of probiotics for the treatment of IBD [82]. The relative enrichment of Proteobacteria and specifically γ -Proteobacteria in recent studies emphasizes the potential importance of Gram-negative bacteria in adult and pediatric IBD. Specific components including γ -Proteobacteria were useful for identification of children with IBD; a specific example was the enrichment of the genus *Escherichia/Shigella* in children with ulcerative colitis [83]. These findings are relevant because the genus *Escherichia coli* has been the source of an established probiotic strain in humans, and microbiome research may help steer physicians towards optimal probiotic/disease combinations. Recent advances in terms of understanding functional metagenomics may point to the next generation of probiotics for adult and pediatric IBD. Microbial function was more often affected than microbial composition in a population of adult patients with Crohn's disease and ulcerative colitis [79]. Major shifts in oxidative stress were identified in the adult IBD state, and relative reductions were identified in genes and pathways involved in carbohydrate metabolism and amino acid biosynthesis in IBD [79]. These differences in terms of metagenomic capacity may be important for the rational selection of probiotics supplying 'missing' functions or factors that interfere with disease-promoting pathways in the microbiome.

Microbiome, Probiotics and Atopic Disease

Microbiome of the Human Skin and Atopy

The human skin contains several dominant bacterial genera across different sites, including *Corynebacterium*, *Eubacterium*, *Propionibacterium*, *Staphylococcus* and *Streptococcus* [84], and one dominant fungal genus *Malassezia* [85]. Focused studies on specific body compartments have highlighted key features of colonization in healthy individuals. *Corynebacterium* was the most common bacterial genus in the anterior nares [86], and the human pathogen *Staphylococcus aureus* was present in a substantial proportion (36%) of healthy human subjects [87]. Relative differences in composition and function of bacterial communities on the human skin may explain different patterns of atopic diseases involving the skin and airways. In cases of atopic dermatitis, staphylococci including *S. aureus* and *S. epidermidis* populations appear to 'bloom' and contribute to disease flares and relapse at specific skin sites [87]. Perhaps, the topical application of probiotics or skin bacterial communities that suppress pathogen 'blooms' on specific body surfaces may help prevent or mitigate these atopic disease flares in the fu-

ture. Unfortunately for patients, the identification of probiotic strains that bestow beneficial effects on the human skin has not been defined, and such applications in dermatology await further investigation. Past limited successes with oral probiotics and amelioration of atopic skin disease features in children [88, 89] have generated optimism for the potential roles of oral or topical probiotics in the treatment of atopy. However, this enthusiasm has been tempered by the realization that many gaps exist in our knowledge of the skin microbiome, probiotics and pediatric allergic diseases, and no single probiotic strain can be recommended at this time [90].

Relative differences in composition and function of bacterial communities on the human skin may explain different patterns of atopic diseases involving the skin and airways.

Microbiome of the Airways: Asthma and Atopy

Alterations in the human microbiome and pathogens have been implicated as possible causes of asthma and potential triggers of asthmatic episodes. In a study of healthy children and children with asthma, there were no significant shifts in bacterial phyla detected in the respiratory tract, and the predominant phyla in both groups included Bacteroidetes, Firmicutes and Proteobacteria [91]. Whereas healthy children were characterized by the genera *Prevotella*, *Streptococcus*, *Veillonella* and *Fusobacterium*, the genus *Haemophilus* was relatively abundant in the asthmatic group. Within the genus *Haemophilus*, the pathogenic species *Haemophilus influenzae* was previously implicated as a potential trigger of asthmatic episodes. A pediatric study from Ecuador yielded intriguing results with respect to the airways microbiome; treatment of respiratory illnesses differs greatly in Ecuador from the standard of care in the United States. Oropharyngeal swabs were obtained from wheezing and healthy infants, and all patients had minimal exposure to antibiotics and no exposure to inhaled steroids [92]. The overall bacterial community in the study population (healthy and wheezing children) consisted primarily of the bacterial phyla Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes and Fusobacterium in order of predominance. The most common genera isolated were consistent with a prior study [91], with most bacteria belonging to *Streptococcus*, *Veillonella*, *Atopobium* and *Prevotella*. In the

Fig. 3. Probiotics modulate key signaling pathways in intestinal epithelial cells. Different probiotic species and strains target distinct signaling pathways in the gut mucosa. These pathways may be exploited to select specific probiotics based on defined mechanisms of action affecting the intestinal microbiome and the mucosal immune system (adapted with permission from MacMillan Publishers Ltd. on behalf of Cander Research UK from Thomas and Versalovic [93]).

wheezing group, a greater frequency of the bacterial genera *Neisseria*, *Corynebacterium*, *Staphylococcus*, *Actinomyces* and *Haemophilus* were identified [92]. Possibly, these differences in the microbiome of the airways account for differences in the susceptibility to asthma or symptoms such as wheezing in the context of immune dysregulation. To reiterate, although future probiotic strategies may be applied by oral or inhaled administration, many gaps exist in our knowledge about the microbiome of the airways, effective probiotics and effects on asthma and allergic diseases [90].

Summary and Future Directions

Steady advances in our knowledge of the composition and function of the human microbiome at multiple body sites including the gut, skin and airways should contribute to our understanding of mechanisms of probiosis. Although our knowledge about microbial composition in *Homo sapiens* is progressing rapidly, many gaps exist in our knowledge about the functional capacity and metabolic machinery of the human microbiome. Although more studies are needed, probiotics appear capable of affecting the composition and function of the microbiome. Effects on function are likely to be more important in the short term (hours to days) following initial administration. Probiotics have yielded beneficial effects in numerous studies in the context of different disease states in pediatric gastroenterology. These disease states include NEC, antibiotic-associated diarrhea and colitis, acute gas-

troenteritis and IBS. An enhanced understanding of the effects of probiotics on the microbiome should facilitate selection of optimal probiotic strains for specific diseases.

Future directions include studies of effects of specific probiotic strains on the human microbiome. Such studies may include experiments evaluating changes in microbial composition using in vitro model systems, 'humanized' animal models containing human-associated bacteria, and clinical studies determining effects on human-associated bacterial communities following probiotics administration. Changes in composition could be extended to evaluation of changes in microbiome function and the related changes in specific metabolic pathways caused by individual probiotic strains. By understanding how probiotic strains alter specific functions of the human microbiome at different body sites, probiotic strain selection may be optimized for specific disease states (fig. 3). As we proceed into the era of metagenomic medicine, patients may be tested for their own microbial compositional and functional features so that probiotics may be customized and tailored to the disease state and the individual patient. The fusion of the microbiome with microbe-based therapies in medicine will advance the causes of holistic and personalized medicine.

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References

- Pirisi A: Phage therapy – advantages over antibiotics? *Lancet* 2000;356:1418.
- Keen EC: Phage therapy: concept to cure. *Front Microbiol* 2012;3:238.
- Fleming A: On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzae* (reprinted from *Br J Exp Pathol*). *Clin Infect Dis* 1980;2:129–139.
- Metchnikoff I: *The Prolongation of Life: Optimistic Studies*. New York, Springer Publishing Company, 1908.
- Lilly DM, Stillwell RH: Probiotics: growth-promoting factors produced by microorganisms. *Science* 1965;147:747–748.
- Parker RB: Probiotics, the other half of the antibiotic story. *Anim Nutr Health* 1974;29:4–8.
- Fuller R: Probiotics in man and animals. *J Appl Bacteriol* 1989;66:365–378.
- FAO/WHO: Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Cordoba, 1–4 October, 2001.
- Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, Bonazzi V, McEwen JE, Wetterstrand KA, Deal C, Baker CC, Di Francesco V, Howcroft TK, Karp RW, Lunsford RD, Wellington CR, Belachew T, Wright M, Giblin C, David H, Mills M, Salomon R, Mulkins C, Akolkar B, Begg L, Davis C, Grandison L, Humble M, Khalsa J, Little AR, Peavy H, Pontzer C, Portnoy M, Sayre MH, Starke-Reed P, Zakhari S, Read J, Watson B, Guyer M: The NIH human microbiome project. *Genome Res* 2009;19:2317–2323.
- Johnson CL, Versalovic J: The human microbiome and its potential importance to pediatrics. *Pediatrics* 2012;129:950–960.
- Methe BA, Nelson KE, Pop M, Creasy HH, Giglio MG, Huttenhower C, Gevers D, Petrosino J, Abubucker S, Badger JH, Chinwalla AT, Ear AM, FitzGerald MG, Fulton RS, Hallsworth-Pepin K, Lobos EA, Madupu R, Magrini V, Martin JC, Mitreva M, Muzny DM, Sodergren EJ, Versalovic J, et al: A framework for human microbiome research. *Nature* 2012;486:215–221.
- Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, Creasy HH, Earl AM, FitzGerald MG, Fulton RS, Giglio MG, Hallsworth-Pepin K, Lobos EA, Madupu R, Magrini V, Martin JC, Mitreva M, Muzny D, Sodergren E, Versalovic J, Wollam AM, Worley KC, Wortman JR, Young SK, Zeng Q, Aagaard K, et al: Structure, function and diversity of the healthy human microbiome. *Nature* 2012;486:207–214.
- Zhou Y, Gao H, Mihindukulasuriya KA, La Rosa PS, Wylie KM, Vishnivetskaya T, Podar M, Warner B, Tarr PI, Nelson DE, Fortenberry JD, Holland MJ, Burr SE, Shannon WD, Sodergren E, Weinstock GM: Biogeography of the ecosystems of the healthy human body. *Genome Biol* 2013;14:R1.
- Rappe MS, Giovannoni SJ: The uncultured microbial majority. *Annu Rev Microbiol* 2003;57:369–394.
- Pace NR: Mapping the tree of life: progress and prospects. *Microbiol Mol Biol Rev* 2009;73:565–576.
- Johansson ML, Molin G, Jeppsson B, Nobaek S, Ahrne S, Bengmark S: Administration of different *Lactobacillus* strains in fermented oatmeal soup: in vivo colonization of human intestinal mucosa and effect on the indigenous flora. *Appl Environ Microbiol* 1993;59:15–20.
- De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P: Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* 2010;107:14691–14696.
- Lin A, Bik EM, Costello EK, Dethlefsen L, Haque R, Relman DA, Singh U: Distinct distal gut microbiome diversity and composition in healthy children from Bangladesh and the United States. *PLoS One* 2013;8:e53838.
- Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD: Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011;334:105–108.
- McNulty NP, Yatsunenko T, Hsiao A, Faith JJ, Muegge BD, Goodman AL, Henrissat B, Oozeer R, Cools-Portier S, Gobert G, Chervaux C, Knights D, Lozupone CA, Knight R, Duncan AE, Bain JR, Muehlbauer MJ, Newgard CB, Heath AC, Gordon JI: The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. *Sci Transl Med* 2011;3:106ra.
- Gibson GR, Beatty ER, Wang X, Cummings JH: Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 1995;108:975–982.
- Bode L: Human milk oligosaccharides: every baby needs a sugar mama. *Glycobiology* 2012;22:1147–1162.
- Bullen CL, Willis AT: Resistance of the breast-fed infant to gastroenteritis. *Br Med J* 1971;3:338–343.
- Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH: Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* 1994;344:1046–1049.
- Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, Kau AL, Rich SS, Concannon P, Mychalekcy JC, Liu J, Hout E, Li JV, Holmes E, Nicholson J, Knights D, Ursell LK, Knight R, Gordon JI: Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science* 2013;339:548–554.
- Santos F, Vera JL, van der Heijden R, Valdez G, de Vos WM, Sesma F, Hugenholtz J: The complete coenzyme B12 biosynthesis gene cluster of *Lactobacillus reuteri* CRL1098. *Microbiology* 2008;154:81–93.
- Saulnier DM, Santos F, Roos S, Mistretta TA, Spinler JK, Molenaar D, Teusink B, Versalovic J: Exploring metabolic pathway reconstruction and genome-wide expression profiling in *Lactobacillus reuteri* to define functional probiotic features. *PLoS One* 2011;6:e18783.
- Dairi T: Menaquinone biosyntheses in microorganisms. *Methods Enzymol* 2012;515:107–122.
- Thomas CM, Hong T, van Pijkeren JP, Hemarajata P, Trinh DV, Hu W, Britton RA, Kalkum M, Versalovic J: Histamine derived from probiotic *Lactobacillus reuteri* suppresses TNF via modulation of PKA and ERK signaling. *PLoS One* 2012;7:e31951.
- De Biase D, Pennacchietti E: Glutamate decarboxylase-dependent acid resistance in orally acquired bacteria: function, distribution and biomedical implications of the gadBC operon. *Mol Microbiol* 2012;86:770–786.
- van Zanten GC, Knudsen A, Roytjo H, Forssten S, Lawther M, Blennow A, Lahtinen SJ, Jakobsen M, Svensson B, Jespersen L: The effect of selected synbiotics on microbial composition and short-chain fatty acid production in a model system of the human colon. *PLoS One* 2012;7:e47212.
- Konieczna P, Groeger D, Ziegler M, Frei R, Ferstl R, Shanahan F, Quigley EM, Kiely B, Akdis CA, O'Mahony L: *Bifidobacterium infantis* 35624 administration induces Foxp3 T regulatory cells in human peripheral blood: potential role for myeloid and plasmacytoid dendritic cells. *Gut* 2012;61:354–366.
- Ewaschuk JB, Walker JW, Diaz H, Madsen KL: Bioproduction of conjugated linoleic acid by probiotic bacteria occurs in vitro and in vivo in mice. *J Nutr* 2006;136:1483–1487.
- Maragkoudakis PA, Mountzouris KC, Rosu C, Zoumpoulou G, Papadimitriou K, Dalaka E, Hadjipetrou A, Theofanous G, Strozzi GP, Carlini N, Zervas G, Tsakalidou E: Feed supplementation of *Lactobacillus plantarum* PCA 236 modulates gut microbiota and milk fatty acid composition in dairy goats – a preliminary study. *Int J Food Microbiol* 2010;141(suppl 1):S109–S116.
- Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO: Development of the human infant intestinal microbiota. *PLoS Biol* 2007;5:e177.
- Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI: Human gut microbiome viewed across age and geography. *Nature* 2012;486:222–227.

- 37 Mai V, Young CM, Ukhanova M, Wang X, Sun Y, Casella G, Theriaque D, Li N, Sharma R, Hudak M, Neu J: Fecal microbiota in premature infants prior to necrotizing enterocolitis. *PLoS One* 2011;6:e20647.
- 38 Sullivan S, Schanler RJ, Kim JH, Patel AL, Trawoger R, Kiechl-Kohlendorfer U, Chan GM, Blanco CL, Abrams S, Cotten CM, Laroia N, Ehrenkranz RA, Dudell G, Cristofalo EA, Meier P, Lee ML, Rechtman DJ, Lucas A: An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. *J Pediatr* 2010;156:562–567.e1.
- 39 Fallon EM, Nehra D, Potemkin AK, Gura KM, Simpser E, Compher C, Puder M: ASPEN clinical guidelines: nutrition support of neonatal patients at risk for necrotizing enterocolitis. *J Parenter Enteral Nutr* 2012;36:506–523.
- 40 Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T: Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2011;3:CD005496.
- 41 Mihatsch WA, Braegger CP, Decsi T, Kolacek S, Lanzinger H, Mayer B, Moreno LA, Pohlandt F, Puntis J, Shamir R, Stadtmuller U, Szajewska H, Turk D, van Goudoever JB: Critical systematic review of the level of evidence for routine use of probiotics for reduction of mortality and prevention of necrotizing enterocolitis and sepsis in preterm infants. *Clin Nutr* 2012;31:6–15.
- 42 Downard CD, Renaud E, St Peter SD, Abdullah F, Islam S, Saito JM, Blakely ML, Huang EY, Arca MJ, Cassidy L, Aspelund G: Treatment of necrotizing enterocolitis: an American Pediatric Surgical Association outcomes and clinical trials committee systematic review. *J Pediatr Surg* 2012;47:2111–2122.
- 43 Guillet R, Stoll BJ, Cotten CM, Gantz M, McDonald S, Poole WK, Phelps DL: Association of H2-blocker therapy and higher incidence of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2006;117:e137–e142.
- 44 Terrin G, Passariello A, De Curtis M, Manguso F, Salvia G, Lega L, Messina F, Paludetto R, Canani RB: Ranitidine is associated with infections, necrotizing enterocolitis, and fatal outcome in newborns. *Pediatrics* 2012;129:e40–e45.
- 45 Kim J, Smathers SA, Prasad P, Leckerman KH, Coffin S, Zaoutis T: Epidemiological features of *Clostridium difficile*-associated disease among inpatients at children's hospitals in the United States, 2001–2006. *Pediatrics* 2008;122:1266–1270.
- 46 Gorbach SL, Chang TW, Goldin B: Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. *Lancet* 1987;2:1519.
- 47 Gorbach SL: Probiotics and gastrointestinal health. *Am J Gastroenterol* 2000;95(suppl 1):S2–S4.
- 48 Guandalini S: Probiotics for prevention and treatment of diarrhea. *J Clin Gastroenterol* 2011;45(suppl):S149–S153.
- 49 Preidis GA, Versalovic J: Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. *Gastroenterology* 2009;136:2015–2031.
- 50 Goldenberg JZ, Ma SS, Saxton JD, Martzen MR, Vandvik PO, Thorlund K, Guyatt GH, Johnston BC: Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. *Cochrane Database Syst Rev* 2013;5:CD006095.
- 51 Preidis GA, Saulnier DM, Blutt SE, Mistretta TA, Riehle KP, Major AM, Venable SF, Barish JP, Finegold MJ, Petrosino JF, Guerrant RL, Conner ME, Versalovic J: Host response to probiotics determined by nutritional status of rotavirus-infected neonatal mice. *J Pediatr Gastroenterol Nutr* 2012;55:299–307.
- 52 Chang JY, Antonopoulos DA, Kalra A, Tonelli A, Khalife WT, Schmidt TM, Young VB: Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*-associated diarrhea. *J Infect Dis* 2008;197:435–438.
- 53 Mattila E, Uusitalo-Seppala R, Wuorela M, Lehtola L, Nurmi H, Ristikankare M, Moilanen V, Salminen K, Seppala M, Mattila PS, Anttila VJ, Arkkila P: Fecal transplantation, through colonoscopy, is effective therapy for recurrent *Clostridium difficile* infection. *Gastroenterology* 2012;142:490–496.
- 54 Thomas DW, Greer FR: Probiotics and prebiotics in pediatrics. *Pediatrics* 2010;126:1217–1231.
- 55 Piescik-Lech M, Shamir R, Guarino A, Szajewska H: Review article: the management of acute gastroenteritis in children. *Aliment Pharmacol Ther* 2013;37:289–303.
- 56 Di Cagno R, De Angelis M, De Pasquale I, Ndagijimana M, Vernocchi P, Ricciuti P, Gagliardi F, Laghi L, Crecchio C, Guerzoni ME, Gobetti M, Francavilla R: Duodenal and faecal microbiota of celiac children: molecular, phenotype and metabolome characterization. *BMC Microbiol* 2011;11:219.
- 57 Palma GD, Capilla A, Nova E, Castillejo G, Varea V, Pozo T, Garrote JA, Polanco I, Lopez A, Ribes-Koninckx C, Marcos A, Garcia-Novo MD, Calvo C, Ortigosa L, Pena-Quintana L, Palau F, Sanz Y: Influence of milk-feeding type and genetic risk of developing coeliac disease on intestinal microbiota of infants: the PROFICEL study. *PLoS One* 2012;7:e30791.
- 58 Sellitto M, Bai G, Serena G, Fricke WF, Sturgeon C, Gajer P, White JR, Koenig SS, Sakamoto J, Boothe D, Gicquelais R, Kryszak D, Puppa E, Catassi C, Ravel J, Fasano A: Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants. *PLoS One* 2012;7:e33387.
- 59 Fernandez-Feo M, Wei G, Blumenkranz G, Dewhirst FE, Schuppan D, Oppenheim FG, Helmerhorst EJ: The cultivable human oral gluten-degrading microbiome and its potential implications in coeliac disease and gluten sensitivity. *Clin Microbiol Infect* 2013;19:E386–E394.
- 60 Saulnier DM, Riehle K, Mistretta TA, Diaz MA, Mandal D, Raza S, Weidler EM, Qin X, Coarfa C, Milosavljevic A, Petrosino JF, Highlander S, Gibbs R, Lynch SV, Shulman RJ, Versalovic J: Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology* 2011;141:1782–1791.
- 61 Rajilic-Stojanovic M, Biagi E, Heilig HG, Kajander K, Kekkonen RA, Tims S, de Vos WM: Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology* 2011;141:1792–1801.
- 62 Jeffery IB, O'Toole PW, Ohman L, Claesson MJ, Deane J, Quigley EM, Simren M: An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut* 2012;61:997–1006.
- 63 Carroll IM, Ringel-Kulka T, Siddle JP, Ringel Y: Alterations in composition and diversity of the intestinal microbiota in patients with diarrhea-predominant irritable bowel syndrome. *Neurogastroenterol Motil* 2012;24:521–530, e248.
- 64 Chassard C, Dapoigny M, Scott KP, Crouzet L, Del'homme C, Marquet P, Martin JC, Pickering G, Ardid D, Eschaliere A, Dubray C, Flint HJ, Bernalier-Donadille A: Functional dysbiosis within the gut microbiota of patients with constipated-irritable bowel syndrome. *Aliment Pharmacol Ther* 2012;35:828–838.
- 65 Rigsbee L, Agans R, Shankar V, Kenche H, Khamis HJ, Michail S, Paliy O: Quantitative profiling of gut microbiota of children with diarrhea-predominant irritable bowel syndrome. *Am J Gastroenterol* 2012;107:1740–1751.
- 66 O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EM: Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005;128:541–551.
- 67 Whorwell PJ, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, Kiely B, Shanahan F, Quigley EM: Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. *Am J Gastroenterol* 2006;101:1581–1590.
- 68 Guandalini S, Magazzu G, Chiaro A, La Balustra V, Di Nardo G, Gopalan S, Sibal A, Romano C, Canani RB, Lionetti P, Setty M: VSL#3 improves symptoms in children with irritable bowel syndrome: a multicenter, randomized, placebo-controlled, double-blind, crossover study. *J Pediatr Gastroenterol Nutr* 2010;51:24–30.

- 69 Bausserman M, Michail S: The use of *Lactobacillus* GG in irritable bowel syndrome in children: a double-blind randomized control trial. *J Pediatr* 2005;147:197–201.
- 70 Mayer EA: Gut feelings: the emerging biology of gut-brain communication. *Nat Rev Neurosci* 2011;12:453–466.
- 71 Cryan JF, Dinan TG: Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* 2012;13:701–712.
- 72 Diaz Heijtz R, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S: Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci USA* 2011;108:3047–3052.
- 73 Amaral FA, Sachs D, Costa VV, Fagundes CT, Cisalpino D, Cunha TM, Ferreira SH, Cunha FQ, Silva TA, Nicoli JR, Vieira LQ, Souza DG, Teixeira MM: Commensal microbiota is fundamental for the development of inflammatory pain. *Proc Natl Acad Sci USA* 2008;105:2193–2197.
- 74 Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF: Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci USA* 2011;108:16050–16055.
- 75 Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, Verdu EF, Collins SM: The intestinal microbiota affect central levels of brain-derived neurotrophic factor and behavior in mice. *Gastroenterology* 2011;141:599–609, e1–e3.
- 76 Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, Guyonnet D, Legrain-Raspaud S, Trotin B, Naliboff B, Mayer EA: Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology* 2013;144:1394–1401.e4.
- 77 Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR: Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007;104:13780–13785.
- 78 Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Dore J: Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflamm Bowel Dis* 2009;15:1183–1189.
- 79 Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, Xavier RJ, Huttenhower C: Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol* 2012;13:R79.
- 80 Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M: Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000;119:305–309.
- 81 Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M: Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 2003;124:1202–1209.
- 82 Whelan K, Quigley EM: Probiotics in the management of irritable bowel syndrome and inflammatory bowel disease. *Curr Opin Gastroenterol* 2013;29:184–189.
- 83 Papa E, Docktor M, Smillie C, Weber S, Preheim SP, Gevers D, Giannoukos G, Ciulla D, Tabbaa D, Ingram J, Schauer DB, Ward DV, Korzenik JR, Xavier RJ, Bousvaros A, Alm EJ: Non-invasive mapping of the gastrointestinal microbiota identifies children with inflammatory bowel disease. *PLoS One* 2012;7:e39242.
- 84 Gao Z, Perez-Perez GI, Chen Y, Blaser MJ: Quantitation of major human cutaneous bacterial and fungal populations. *J Clin Microbiol* 2010;48:3575–3581.
- 85 Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, Schoenfeld D, Nomicos E, Park M, Becker J, Benjamin B, Blakesley R, Bouffard G, Brooks S, Coleman H, Dekhtyar M, Gregory M, Guan X, Gupta J, Han J, Hargrove A, Ho SL, Johnson T, Legaspi R, Lovett S, Madero Q, Masiello C, Maskeri B, McDowell J, Montemayor C, Mullikin J, Riebow N, Schandler K, Schmidt B, Sison C, Stantiprop M, Thomas J, Thomas P, Vemulapalli M, Young A, Kong HH, Segre JA: Topographic diversity of fungal and bacterial communities in human skin. *Nature* 2013, E-pub ahead of print.
- 86 Grice EA, Segre JA: The skin microbiome. *Nat Rev Microbiol* 2011;9:244–253.
- 87 Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, Nomicos E, Polley EC, Komarow HD, Murray PR, Turner ML, Segre JA: Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 2012;22:850–859.
- 88 Majamaa H, Isolauri E: Probiotics: a novel approach in the management of food allergy. *J Allergy Clin Immunol* 1997;99:179–185.
- 89 Abrahamsson TR, Jakobsson T, Bottcher MF, Fredrikson M, Jenmalm MC, Bjorksten B, Oldaeus G: Probiotics in prevention of IgE-associated eczema: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2007;119:1174–1180.
- 90 Fiocchi A, Burks W, Bahna SL, Bielory L, Boyle RJ, Cocco R, Dreborg S, Goodman R, Kuitunen M, Haahtela T, Heine RG, Lack G, Osborn DA, Sampson H, Tannock GW, Lee BW: Clinical use of probiotics in pediatric allergy (CUPPA): a World Allergy Organization position paper. *World Allergy Organ J* 2012;5:148–167.
- 91 Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, Davies J, Ervine A, Poulter L, Pachter L, Moffatt MF, Cookson WO: Disordered microbial communities in asthmatic airways. *PLoS One* 2010;5:e8578.
- 92 Cardenas PA, Cooper PJ, Cox MJ, Chico M, Arias C, Moffatt MF, Cookson WO: Upper airways microbiota in antibiotic-naïve wheezing and healthy infants from the tropics of rural Ecuador. *PLoS One* 2012;7:e46803.
- 93 Thomas CM, Versalovic J: Probiotics-host communication: modulation of signaling pathways in the intestine. *Gut Microbes* 2010;1:148–163.