The interpersonal and intrapersonal diversity of human-associated microbiota in key body sites

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The human body harbors 10 to 100 trillion microbes, mainly bacteria in our gut, which greatly outnumber our own human cells. This bacterial assemblage, referred to as the human microbiota, plays a fundamental role in our well-being. Deviations from healthy microbial compositions (dysbiosis) have been linked with important human diseases, including inflammation-linked disorders, such as allergies, obesity, and inflammatory bowel disease. Characterizing the temporal variations and community membership of the healthy human microbiome is critical to accurately identify the significant deviations from normality that could be associated with disease states. However, the diversity of the human microbiome varies between body sites, between patients, and over time. Environmental differences have also been shown to play a role in shaping the human microbiome in different cultures, requiring that the healthy human microbiome be characterized across life spans, ethnicities, nationalities, cultures, and geographic locales. In this article we summarize our knowledge on the microbial composition of the 5 best-characterized body sites (gut, skin, oral, airways, and vagina), focusing on interpersonal and intrapersonal variations and our current understanding of the sources of this variation. (J Allergy Clin Immunol 2012;129:1204-8.)

Key words: *Human microbiome, microbial diversity, temporal variation, 16S rRNA sequencing*

Abbreviations used
BV: Bacterial vaginosis
CF: Cystic fibrosis
GIT: Gastrointestinal tract
OTU: Operational taxonomic unit
QIIME: Quantitative Insights Into Microbial Ecology

The human body is colonized by trillions of microbial cells, collectively referred to as the microbiota, whereas the combination of these microbial cells and their corresponding suite of genes is defined as the microbiome.[1] The introduction of sample barcoding,[2-4] the decreasing cost of next-generation sequencing technologies,[5,6] improvements in bioinformatics tools,[7,8] and online databases[9-11] have allowed researchers to categorize what microbes live in and on the human body and to define the similarities and differences between human microbiota. The first human-associated microbial studies quickly discovered the high degree of variability in the microbiota between subjects[12-16]; these studies were rapidly extended to show that variability is also high within subjects both between different body sites and over time within one body site.[17-19] More recent studies have been able to sample the microbiota densely over time[20] and in large cohorts.[21] Efforts like the Human Microbiome Project[1,21,22] are beginning to elucidate the variations found in healthy adult microbial communities. It is uncertain at this point whether the differences in microbiota seen in many disease states are a symptom of the disease or a contributing factor. However, defining a healthy microbial state is a critical step for discovering how variations in the microbiome can contribute to or cause a wide range of diseases.[23]

TECHNIQUES FOR MICROBIAL COMMUNITY ANALYSIS

Sample barcoding coupled with high-throughput sequencing has allowed microbiologists to study microbial communities at an unprecedented depth over the past few years. By identifying each sample with a unique nucleotide barcode added to the PCR primer used to amplify microbial 16S ribosomal RNA, samples can be pooled together and run at the same time on a high-throughput sequencer. The sequences can then be imported into a number of software pipelines for microbial analysis, including mothur,[24] W.A.T.E.R.S,[25] and Quantitative Insights Into Microbial Ecology (QIIME).[7] The prevalence of cloud computing, including the Amazon Web Services Elastic Compute Cloud, means that anyone with Internet access can connect to a supercomputer and analyze hundreds of millions of microbial sequences with minimal upfront costs (eg, renting a computer with 8 processors and 68GB of RAM from Amazon Web Services costs about USD$2.00 per hour).

The open-source software QIIME (www.qiime.org; pronounced "chime") takes users from their raw sequence data, through quality filtering and other initial analysis steps, through alpha and beta diversity analyses (defined below), and ultimately through publication-quality graphics. An early step in microbial community analysis workflows is clustering of sequence reads

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into operational taxonomic units (OTUs). An OTU cluster is usually defined on the basis of sequence similarity: frequently, reads that are greater than or equal to 97% identical to one another are clustered into an OTU. This is primarily done for computational efficiency: compute-intensive downstream steps, such as assigning taxonomy to sequences, can be performed on a single representative sequence from an OTU rather than on many nearly identical sequences.

Ultimately, many researchers are interested in understanding the microbial diversity of their samples: main alpha (or within-sample) diversity and beta (or between-sample) diversity. Alpha diversity can be measured, for example, as the number of unique OTUs found within a given community. Beta diversity, on the other hand, is frequently measured by computing pairwise UniFrac distances (the fraction of branch length in a phylogenetic tree that is unique to either sample[26]) between samples. Communities that are very similar phylogenetically result in low UniFrac scores, whereas dissimilar communities produce high UniFrac scores. UniFrac distances between many samples can be represented in a distance matrix, and that distance matrix can be summarized and visualized in 3-dimensional space by using principal coordinates analysis, a dimensionality reduction technique that summarizes the distances between samples in a scatter plot in which points (representing samples) that are more distant from one another are more dissimilar.

GUT MICROBIOTA

Effect of diet in defining gut microbial communities

The human gut represents an important reservoir of bacteria that has been shown to play an important role in human health, including priming the host immune system and possibly causing disease states through microbial community dysbiosis.[27,28] Diet is the most powerful influence on gut microbial communities in healthy human subjects.[29-32] A study of human subjects and 59 other mammals revealed clustering in which the effects of diet (carnivorous, omnivorous, or herbivorous) in most cases outweighed host phylogeny.[30] Recent analysis suggested that the gut microbiota could be classified as belonging to one of 3 principal variants, or "enterotypes," defined by a dominant presence of *Bacteroides*, *Prevotella*, or *Ruminococcus* species.[31] However, these enterotypes seem to be more microbial gradients than discrete communities and can largely be explained by long-term dietary intake: *Bacteroides* species were prevalent with long-term protein and animal fat diets, whereas *Prevotella* species were associated with long-term carbohydrate diets.[32]

Twin studies have also been influential in elucidating the role that the environment plays in defining the gut microbiome. One study compared monozygotic and dizygotic twins living in South Korea and the United States, including pairs of European and African descent.[33] Alpha diversity was not significantly different between the Korean and US cohorts, demonstrating that one cohort did not contain a greater number of OTUs than the other. UniFrac distances between the 2 groups revealed that the phylogenetic composition of the gut community in the Korean cohort was significantly different from that in the US cohort (including the African American and European American subgroups). Family-level taxa that discriminated between the Korean and US cohorts included Bacteroidaceae, Enterococcaceae, Lactobacillaceae, Leuconostocaceae, Prevotellaceae, Rikenellaceae, Ruminococaceae, Streptococcaceae, and Veillonellaceae.[33]

Altered microbiota in obese subjects

Differences between the South Korean and US cohorts decreased when comparing obese subjects across the 2 groups.[33] Principal coordinates analysis revealed that distinct clustering of South Korean and US cohorts was greater when comparing only lean subjects than when comparing lean and obese subjects. This suggests that obesity is masking some of the dietary and environmental factors between these 2 groups. Twin studies reveal that one difference between the gut communities of lean and obese subjects might be related to reduced alpha diversity, which is commonly seen in obese patients relative to lean patients.[17,33] Obesity has also been observed to correlate with several phylum-level bacterial changes, including decreased Bacteroidetes and increased Firmicutes levels within subjects on a weight-reduction diet,[34] although the pattern between subjects in lean and obese populations appears to be more complex.[17,35,36]

Biogeography of the human gastrointestinal tract

Recent studies have evaluated human-associated microbiota along the length of the gastrointestinal tract (GIT). Work by Stearns et al[37] sampled the mouth, stomach, duodenum, colon, and stool from 2 healthy male and 2 healthy female subjects. They found that the mouth harbored the greatest phylogenetic diversity, the stomach had the lowest diversity, and diversity increased down the GIT from the stomach to the stool. Twenty-five OTUs at various taxonomic levels were present in every sampling site of every subject, including *Faecalibacterium* species, TM7, and *Streptococcus* species. As is typical in human microbiota studies, clustering was seen between sample sites along the GIT rather than clustering based on subject or sex.[37]

Interestingly, constrained ordination methods have been used to demonstrate that differences exist between male and female microbial communities.[38] In this study male subjects ($n = 5$) clustered more closely together than female subjects ($n = 5$) and were enriched in *Faecalibacterium prausnitzii*, *Bifidobacterium* species, and *Bacteroides*, *Clostridium*, *Enterococcus*, and *Prevotella* species. Female subjects had enhanced signals from *Streptococcus*, *Veillonella*, *Mannheimia*, and *Ruminococcus* species relative to male subjects.[38] The GIT has also been shown to have a biogeographic distribution of microbes. Using numeric ecology methods to remove intersubject variability, one study suggests that there might be evidence of microbial gradients along the GIT. For example, levels of Enterobacteriaceae were shown to increase toward the distal end of the GIT (the sigmoid colon and rectum), whereas *Streptococcus* species, Comamonadaceae, *Enterococcus* species, and *Corynebacterium* species had increased abundance in the proximal end of the GIT (the cecum and transverse colon).[38]

SKIN MICROBIOTA

The skin represents an interesting human habitat in which lifestyle and environmental factors shape the microbial community of different specific body sites. No taxa are ubiquitously present in every subject and body site, although targeted studies reveal that specific body sites are generally dominated by certain defining taxa: *Bacteroidetes* species in the GIT, *Lactobacillus* species in the vagina, *Streptococcus* species in the oral cavity, and *Propionibacterium* species in the retroauricular crease.[16-19,22,39] The human skin is mainly comprised of

Actinobacteria, Proteobacteria, and Firmicutes, with 1 study finding that more than 90% of the microbiota of the forearm belonged to these phyla.[40]

A hallmark of human skin microbiota communities is high diversity and high interpersonal variation. Costello et al[19] found that skin sites, including the palms, fingers, and forearms, had greater phylogenetic diversity than communities in the gut, external auditory canal, or oral cavity. The volar forearms of different subjects were found to only share 2% of species-level OTUs,[40] whereas the hands share 13% of OTUs.[14] Estimates of species-level OTUs for skin sites include 246 for the volar forearm,[40] more than 150 for the palms of the hands,[14] and 113 for the inner elbow.[15] More than 50% of sequences obtained from arm skin sites belong to *Propionibacterium*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, and *Lactobacillus* species.[14,40] Diversity among skin sites of the same subject is also high. One study found that of the total 48 species-level OTUs found on the forearms, on average, only 13.5 were shared between the left and right forearms on the same subjects, representing 67.9% of clones.[40] Similarly, the left and right hands of the same subjects were shown to share 17% of OTUs, with diversity more than 3-fold greater than that of the forearm or elbow.[14] Although the skin does harbor hundreds of unique OTUs, our current level of sequencing is likely not revealing all OTUs present.[15]

Fungal microbiota of the human forearm have also been explored in healthy patients and in patients with psoriasis.[41] Five healthy patients and 3 patients with psoriasis had their forearms sampled, and the 18s rDNA was sequenced to detect eukaryotes. Most sequences obtained resembled *Malassezia* at the genus level, and differences in the communities of patients with psoriasis were noted in a majority of cases. One limitation to this study was the low level of 18s rDNA sequences present in GenBank at the time. As databases improve, our knowledge of the presence and diversity of eukaryotic microbiota on the human body will continue to increase.

ORAL MICROBIOTA

The microbial community of the oral cavity is unique compared with that of other body habitats and contains high variability between subjects.[19,42-44] Different oral sites, including mucosal sites, anaerobic pockets, and teeth, each harbor unique microbial assemblages.[13,45] In a study of the oral communities from 10 healthy adults, 15 bacterial genera were found in all subjects, including the 10 species *Streptococcus oralis*, *Haemophilus parainfluenzae*, *Granulicatella adiacens*, *Veillonella parvula*, *Veillonella dispar*, *Rothia aeria*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, *Prevotella melaninogenica*, and *Capnocytophaga gingivalis*. Despite these similarities, the oral communities had high interpersonal variation, with some oral communities dominated by *Streptococcus* species and others dominated by *Prevotella*, *Neisseria*, *Haemophilus*, or *Veillonella* species. Many species were not shared between subjects for a given genera, including *Neisseria*, *Fusobacterium*, and *Corynebacterium* species.[42]

Another study sampled the oral cavity of 120 subjects from 12 different geographic locations.[43] Although no geographic patterns were apparent, large variations in microbial communities existed between locations. For example, 28% of all sequences derived from the Congo were Enterobacteria, yet this taxa was not found in China, Germany, Poland, Turkey, or California. Larger variations in the microbial communities were found between subjects in the same geographic location than between geographic locations, demonstrating that global geography does not seem to play a role in determining oral microbial communities. The number of genera present (defined as 90% sequence similarity) in any subject ranged from 6 to 30, and 39 previously unreported genera were identified within the human oral cavity.[43]

AIRWAY MICROBIOME

The microbiota associated with the airways has many parallels with other more highly studied parts of the human body. For instance, the distribution of bacteria within the lungs is spatially heterogeneous,[46] exactly the same pattern witnessed in other human body parts, including the skin.[19] Also, distinct microbial communities are present in the airways of diseased patients, including those with cystic fibrosis (CF) and asthma.[46,47] For example, both culturing and deep sequencing have revealed that *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Burkholderia cepacia* are present in the lungs of patients with CF.[46] In patients with CF, *P aeruginosa* can comprise up to 99% of bacterial sequences isolated from the trachea and 51% to 94% of sequences from each lobe of the lung. It has been shown that age can also have a significant effect on the airway microbiota of patients with CF. By using the 16s rRNA PhyloChip to assess the microbiota of patients from 9 to 72 years of age, the study found that older patients with CF had a decrease in bacterial richness, evenness, and diversity while concurrently losing pulmonary function.[48] It would seem that, in patients with CF at least, the highly diverse young airway microbiota is gradually replaced by a less diverse community in which a few members, including *Pseudomonas*, *Staphylococcus*, and *Burkholderia* species, are highly dominant.

The airway microbiota has also been shown to play a role in asthma. A comparison of 65 asthmatic patients taking inhaled corticosteroids with 10 healthy control subjects found that asthmatic patients' airways contained a greater microbial diversity than those of healthy control subjects.[49] Approximately 100 bacterial phyla the presence of which was highly correlated with bronchial hyperresponsiveness were also identified, including the families Sphingomonadaceae, Oxalobacteraceae, Comamonadaceae, Enterobacteriaceae, and Shewanellaceae.[49] In a large adult cohort study, it was found that the prevalence of asthma was negatively correlated with the presence of *Helicobacter pylori*.[50] One hypothesis for the development of asthma postulates that exposure to diverse microbes early in life might have a protective effect against asthma.[51]

VAGINAL MICROBIOME

The vaginal microbial community has long been considered an important defense mechanism against infection.[52-54] Studies that sampled women across different ethnicities, including Caucasian, African American, Hispanic, and Asian women, found that most vaginal communities could be defined by the presence of a dominating *Lactobacillus* species, such as *Lactobacillus iners*, *Lactobacillus crispatus*, *Lactobacillus gasseri*, or *Lactobacillus jensenii*.[16,55] The other communities were not dominated by a *Lactobacillus* species but still contained a dominant community of lactic acid–producing microbes.[16] The vaginal communities of Asian and Caucasian women were most often dominated by

lactic acid–producing *Lactobacillus* species than those of Hispanic and African American women, possibly causing the lower vaginal pH levels found in Asian and Caucasian women. Bacterial vaginosis (BV) results in a significant community shift from healthy communities and negative health consequences.[16] Twenty-nine percent of species-level OTUs were shared between healthy and BV-positive women because BV-positive communities were characterized by decreases in *Lactobacillus* species and increases in *Gardnerella*, *Atopobium*, *Megasphaera*, *Eggerthella*, *Aerococcus*, *Leptotrichia/Sneathia*, *Prevotella*, and *Papillibacter* species.[56]

TEMPORAL VARIATION OF HUMAN MICROBIOME DIVERSITY

Development of the human microbiome is a dynamic process, with different life stages displaying notable differences in terms of diversity and variation.[57] Variation between human body sites in adults is stable over time, but different body sites converge on the healthy adult microbiome through different trajectories. For example, newborns are rapidly colonized by the microbial communities of the mother's vagina if delivered vaginally or by microbes resembling skin if delivered during cesarean birth.[58] The child's gut microbiota acquires phylogenetic diversity linearly, resembling that of a healthy adult by 2 years of age.[59] However, children's oral communities do not resemble those of adults, even at 18 years.[44] The reasons for these differences in colonization are not yet known.

Once developed, stable differences were observed between human body sites over 3 months.[19] Dense sampling over time answered more specific questions about the degree and scale of temporal variation. Caporaso et al[20] studied 2 subjects sampled daily at 4 body sites (tongue, left and right palms, and gut), 1 female subject for 6 months and 1 male subject for 18 months. Variation was greatest in skin communities, followed by gut communities; oral communities were most stable. Interpersonal differences in community composition within body sites were also stable across time. Next, the authors show that despite stability over time, there is a relatively small "temporal core microbiome" at the 97% OTU level. In other words, although communities look relatively similar over time, there are few OTUs that are actually observed at all time points. The size of this temporal core microbiome at the species level correlates with variability: the oral communities have the largest core (approximately 10% of the OTUs are present in 95% of the samples), the gut communities have the next largest core (approximately 5% of OTUs are present in 95% of the samples), and the skin communities have the smallest core (approximately 1% of OTUs are present in 95% of the samples). There appears to be no core temporal microbiome across body sites.

CONCLUSION

The decreasing cost of sequencing has allowed researchers to obtain an unprecedented quantity of 16S rRNA sequencing from larger cohorts sampled more densely over time. These large-scale sampling efforts have corroborated the results of numerous small studies in affirming the large interpersonal variation of the microbiota within a given body habitat and the immense differences found between different body habitats. However, it is possible that some of our original hypothesis on the microbiota developed from experiments with small sample numbers might be overturned as the trend toward ever-larger cohorts continues. The sampling of new populations at increasing depth is continuing to find novel species-level OTUs, demonstrating how our characterization of human-associated microbes is not yet complete. These OTUs can be very important for determining differences between communities and in defining disease states. Although the most recent wave of microbial studies focused on increasing the number of sequences and samples collected, the challenge facing future studies is to increase the clinically relevant information associated with samples to better relate changes in the microbiota to events in human lives. With the continued decrease in the cost of sequencing and the increasing accessibility of the necessary bioinformatics tools, we expect that our understanding of human-associated microbial communities will soon result in novel microbiome-related clinical treatments. We now know what "normal" communities look like to an unprecedented extent: what we need to discover, in a systematic way, is what "diseased" communities look like and which factors can be manipulated to bring them back to the healthy state.

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