The Human Microbiome: Getting Personal

Each of us harbors a unique microbiome, and its characteristics play an important role in differentiating us from one another

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Through reliance on DNA sequencing and associated bioinformatics techniques, we are vastly expanding our understanding of the human microbiome. Each of us harbors a unique microbiome, and its characteristics play an important role in differentiating us from one another. Our microbes contribute to many aspects of our health and our lives, including our metabolism of food, whether we develop cancer, how we behave, and how we respond to medical treatments.

While any two individuals are nearly identical in their genomic composition, they may not share any of the same bacterial species in their gut. Thus, even when temporal variation is accounted for, the human microbiome can be far more "personalized," that is, more different between individuals than within an individual, than the human genome. Here we discuss what is and is not personal about the human microbiome and what that individuality might imply when treating microbiome-associated diseases.

SUMMARY

- **The human microbiome can be far more personalized, that is more different between individuals than within an individual, than the human genome. While the human genome is mostly stable over our lives, our microbiome changes on a daily basis.**
- **Features of the microbiome that differ across individuals include its taxonomic composition and structure, in other words, which taxa are present and at what abundances, and the rate at which the composition and structure change over time.**
- **Our personalized microbiomes reflect our lives. Some features are inherited, with incomplete penetrance, via our genomes; other features develop stochastically due to the timing of our exposure to particular organisms; and still others result from personal choices such as diet or environmental exposures.**
- **Methods for studying the microbiome are being borrowed from other fields and adapted for studying massive data sets.**

Microbial Differences Exceed Those of the Hosts

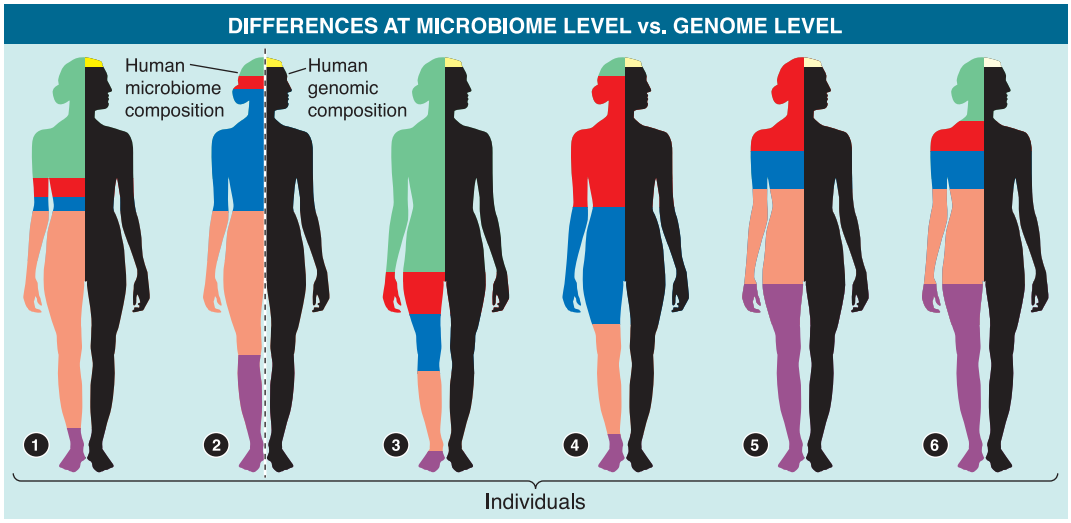
The phylogenetic compositions of microbiomes vary from one individual to another.

Microbial variations within an individual at body sites such as the skin, gut, and oral cavity generally are lower than are those differences between individuals. For example, when two individuals are sampled from the same anatomic site at different times, the differences in microbial communities from the same individual typically are less than are the differences across the individuals, even when those samples are collected years apart.

At some anatomic sites, microbial communities vary less across different sublocations within a person than between two people. For example, bacterial composition of the transcending colon is more similar to that of the sigmoid colon from the same individual than it is to the transcending colon from another individual. However, at very different sites, such as the skin versus the gut, intrapersonal differences become larger than interpersonal differences. For example, the gut and skin microbial communities from one individual differ more from each other than do two gut samples or two skin samples of two different individuals. These differences are so large that they can be used to pick out mislabeled or contaminated samples.

The differences in taxonomic composition of the microbiome between individuals are important. For instance, they are associated with diseases such as colon cancer and inflammatory bowel disease, the severity of autism spectrum disorders, and differences in responses to medical treatments. One striking effect is that of fecal transplantation, in which the microbiome is restored from an aberrant to a more typical state, coupled with remission of clinical symptoms

FIGURE 1



Humans are far more different from each other in their microbiome composition than in their genomic composition. The colors in the left side of each individual represent bacterial phyla, while the colors on the right side indicate host genomic similarity. For the most part, we contain similar phyla living in and on our bodies, but their relative abundances can be drastically different. On the other hand, our genomic composition is nearly identical, with only a small fraction (around 0.1%) differing across individuals.

such as diarrhea following infection with *Clostridium difficile*, as illustrated through studies by Alex Khoruts and colleagues of the University of Minnesota, Minneapolis.

Personal Features of the Human Microbiome

How individualized are our microbiomes? They may be sufficiently unique and stable to be useful in forensics, according to studies by Noah Fierer and Jessica Metcalf at the University of Colorado at Boulder. These, and studies of the personal human microbiome, have focused on microbiome composition and structure—in other words, which taxa are present and in what abundances, and very clearly illustrate that we all differ from each other in those respects.

The variability of the human microbiome, not just its composition, may be both personalized and important for predicting disease susceptibility, according to Pawel Gajer and colleagues at the University of Maryland School of Medicine in Baltimore. For example, the vaginal bacterial communities of some women change more rapidly than do those of other women, and the rate of that change helped to predict the type of bacterial vaginosis that each woman had.

We find that our microbiomes also change at different rates at different body sites. For instance, microbial communities of human skin change more rapidly than do human gut communities, which change more rapidly than do microbial communities within the human mouth—at least for two individuals who we monitored daily for at least six months. This pattern appears to hold up for larger numbers of individuals.

These findings highlight the value of longitudinal studies and a need for more of them. If human microbiome dynamics prove useful for diagnosing or treating disease, data from studies sampling many individuals over longer periods of time will provide better, albeit incomplete, information on which to base medical diagnoses and therapies.

When and Why Does the Personal Microbiome Establish?

Although an individual's microbiome rapidly changes during the first three years of life, personal signatures are evident very early at some

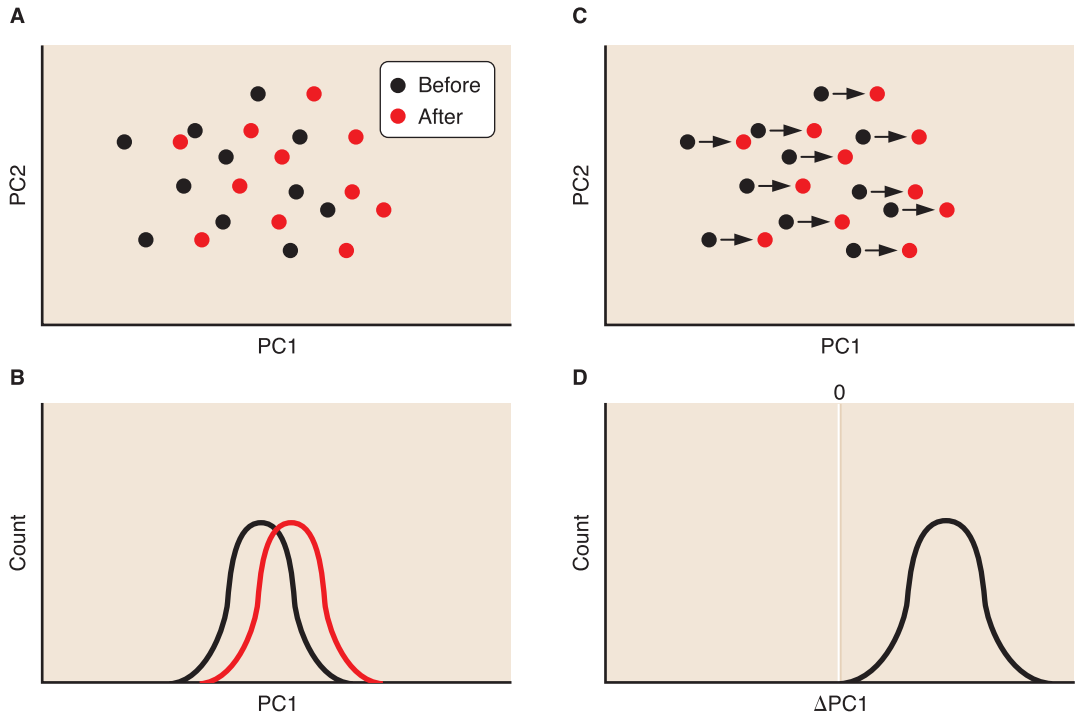
body sites, according to Elizabeth Costello and colleagues at Stanford. These signals typically arise within the first 21 days of life, when skin microbial communities become personalized, or more similar within than between individuals. However, a personalized signature in gut and oral microbiota did not arise over this timeframe.

Several features affect the early composition of an individual's microbiome, including the mode of delivery. For instance, infants who are delivered by cesarean section initially have microbiomes that are similar in composition to the typical microbiota of adult human skin, whereas infants delivered vaginally initially have microbiomes with characteristics of those found in the human vagina. These early signatures diminish over time as the microbiome establishes, and other factors begin to drive its composition and dynamics.

For example, individuals living in households with pets share a higher degree of microbiota similarity with one another than individuals living in households without pets, suggesting that pets may serve as vectors for transferring microbiota between humans. Meanwhile, individuals living near farms tend to have higher bacterial diversity—in other words, more types of bacteria—in their gastrointestinal tracts than do individuals who do not live near farms.

Although the host immune system and perhaps other factors help to set the composition of the microbiome in mice, sharing an environment is a better predictor of microbiome similarity than is genetic relatedness. Taken together, our personalized microbiomes reflect our lives. Some features are inherited, with incomplete penetrance, via our genomes; other features develop stochastically due to the timing of our exposure

FIGURE 2



(A) When comparing whole microbial communities before and after treatment from many subjects, the before/after treatment states are often not immediately evident due to the "personal microbiome" effect. (B) For example, the distributions of Principal Coordinate 1 values for before versus after treatment samples may not be significantly different. (C) However, it is possible to control for the personal microbiome effect by looking for consistent differences associated with treatment. (D) In this example, the PC1 values for all subjects increased with treatment, suggesting a consistent treatment effect across individuals, albeit with a smaller effect size than that of the personal microbiome.

AUTHOR PROFILE

Caporaso: from Computers and Bioinformatics to Fitness and Reading

J. Gregory Caporaso is fond of computers and programming, an interest that took off during his high school statistics and computer graphics courses. Today, Caporaso, 36, assistant professor of biological and computer sciences at Northern Arizona University, focuses on developing high-quality bioinformatics software and analytic methods for working with massive DNA sequence data sets. "The primary application of the software developed in my group is studying how the communities of microorganisms that live in and on our bodies, and cohabit our homes and offices, affect human health, and ultimately how we can use that understanding to improve human health by developing microbiome-based treatments of disease," he says.

The older of two boys, Caporaso grew up in Rockville Centre, N.Y., on Long Island, where his parents owned a printing company. His fascination with computers started in childhood. "My parents were early adopters of Apple computers as they used them for graphic layout and design at work," he says. His access to those computers led him to develop an interest in computer graphics, an interest that his high school art teacher Joan Hochberg, or "Mrs. H," encouraged.

In college at the University of Colorado at Boulder, Caporaso started in the fine arts department but, after a brief interlude as an English major, switched to computer science. After graduating with a B.S. in 2001, he worked in industry developing server-monitoring software, but found it uninspiring. "I though I might be interested in human medicine and started taking some pre-med classes," he says. "While doing that, I also started getting interested in biomedical research, and reached out to several professors to volunteer on their research projects."

He soon met Rob Knight, now a professor in Boulder but then a postdoctoral researcher working with Michael Yarus, also at Boulder. "Mike and Rob were able to pay me part time to do some bioinformatics work in the lab," he says. Knight helped convince him to pursue a research career, and Caporaso chose to go after a Ph.D. in biochemistry, although in practical terms he focused on bioinformatics. "I figured I'd just solve the protein folding problem as my dissertation project," he says. "That didn't exactly work out, but I got a lot of bioinformatics software development experience over the course of my Ph.D., which was instrumental as I moved on to my postdoc." He earned his doctorate in 2009 from the University of Colorado, Denver, and then did postdoctoral research with Knight until 2011, when he joined the Northern Arizona University faculty.

Caporaso makes a point of exercising four or five days a week. He practices CrossFit, and once won the local "Athlete of the Month" award. "Exercise is really important to me," he says. "During grad school and postdoc, this was mostly in the form of running and hiking Colorado mountains. I love the outdoors, and that's motivated me to pick nice places to live." He also enjoys reading fiction, including Alan Moore, the English comics writer. "I also love nonfiction, and Carl Sagan is a personal hero of mine," he says.

Marlene Cimons

Marlene Cimons lives and writes in Bethesda, Md.

to particular organisms; and still others result from personal choices such as diet or environmental exposures.

Some Parts of the Microbiome May Not Be Personalized

Some features of the human microbiome do not appear to be personalized. For instance, some phylum-level taxa are likely to be present universally at specific body sites among healthy adult humans. Although the relative abundance of these phyla likely vary with host diet and other characteristics, some core microbial phyla are essentially universal. For example, the gut bacterial communities of healthy United States residents nearly always contain species belonging to the Bacteroidetes, Firmicutes, and Actinobacteria phyla.

Further, the functional genes encoded by the microbiota appear to be consistent across individuals, despite differences in the composition of microbiota at the genus and species taxonomic levels. While there may be differences in exactly which species are present in the microbiota, the functional repertoire is similar across individual humans. Indeed, it appears likely that each host selects for a collective microbiota that provides a specific set of functions, according to Peter Turnbaugh, Jeffrey Gordon, and their collaborators at Washington University in St. Louis, Mo., who reported those findings in 2009.

Microbiome Data Analysis Requires Multidisciplinary Approaches

Analyzing and interpreting human microbiome data requires multidisciplinary approaches, and our computational and statistical methods have had to change drastically to keep pace with the rapid increase in data. For example, although the process was tedious, a decade ago the National Center for Biotechnology Information (NCBI) BLAST Web server could be used to assign taxonomic origin to 16S rRNA sequences, and it was then possible to tabulate taxa on a per-sample basis in a spreadsheet to perform comparative diversity analyses.

With rapidly expanding microbial DNA sequence data, we are studying orders of magnitude more samples and taxa than in the recent past. This growing volume of data requires new bioinformatics methods to process and interpret, new strategies for handling multiple comparisons, and the use of high-performance computing resources such as cluster and cloud computing. These challenges force us to reevaluate what undergraduate and graduate biology students should be taught. The next generation of microbiologists and microbial ecologists will need to be trained not only in microbiology, but also in the computational techniques that will be necessary to perform their studies.

Analytic Challenges of Measuring Subtle Microbiome Effects

As the field of microbiomics grows, many methods for studying the microbiome are being borrowed from other fields and adapted to studying massive microbial sequence data sets. One current challenge is to understand how to account for the effects of the personalized microbiome when investigating how a novel treatment affects different individuals.

Distinct pretreatment and posttreatment microbial community states might be apparent across individuals when analyzing the changes resulting from a treatment with a large expected effect size, such as fecal microbiota transplants. However, for treatments expected to have a smaller effect size, such as treatment with probiotics, the personal microbiome effect likely will mask the more subtle effect of treatment because treatment may not induce a change in the microbiome that is larger than the typical differences in microbial communities between any two individuals. Approaches for detecting changes in microbial communities that control for the interpersonal microbiome differences are essential because the personal microbiome effect size is so large.

Paired-difference testing can be a useful strategy for overcoming the personal microbiome effect. Instead of comparing all pretreatment and posttreatment samples, one computes the differences only among the observations of interest to detect consistent changes in the microbiota community structure. For example, a particular operational taxonomic unit (OTU) might always decrease in abundance with treatment. However, if the abundance of that OTU in one subject were higher after treatment than its abundance in another subject before treatment, we would not see this pattern using whole-community-profile comparisons of all pretreatment and posttreatment samples, because the abundance distributions would overlap.

Karen Schwarzberg of Northern Arizona University, Scott Kelley of San Diego State University, and their collaborators applied this strategy to analyze the effects of treatment for periodontal disease on the oral microbiome, and identified changes that were apparent only when controlling for the personal microbiome effect. One of the challenges they faced however, was avoiding "false-positive" spurious differences, which are common. When comparing thousands of OTUs across fewer than 100 subjects, some OTUs will change consistently in the same direction following treatment merely by chance, so involving statisticians at the experimental design stage and controlling for multiple comparisons are essential.

Compared to our dynamic microbiomes, our own genomes are mostly static and surprisingly similar across individuals. We are only beginning to address some key questions about how personalized our microbiomes may be. How do they become personalized in the first place? Which microbiome features differ systematically and which at random across individuals? Which features matter? What matters more: the current state of our microbiome, or how much it varies over time? How frequently do we need to sample an individual's microbiome to make useful predictions about health? Before long, routine phys-

icals might include microbiome profiling and monitoring to help address such questions.

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