VIEWPOINT

The microbiome explored: recent insights and future challenges

Martin Blaser, Peer Bork, Claire Fraser, Rob Knight and Jun Wang

Abstract | One of the most exciting scientific advances in recent years has been the realization that commensal microorganisms are not simple 'passengers' in our bodies, but instead have key roles in our physiology, including our immune responses and metabolism, as well as in disease. These insights have been obtained, in part, through the work of large-scale, consortium-driven metagenomic projects. Here, five experts in the field of microbiome research discuss the most surprising and exciting new findings, and outline the future steps that will be necessary to elucidate the numerous roles of the microbiota in human health and disease and to develop viable therapeutic strategies.

Q *The microbiota has been linked with a wide array of host processes; what do you think have been the most surprising and valuable findings to date and why?*

Martin Blaser. In this past year, there have been major advances in our understanding of the role of the microbiome in host physiology. The work of Koren *et al.*[1] provided evidence that the gut microbiota directionally shifts over the course of pregnancy, affecting the metabolism of the mother in ways to adaptively increase nutrients for her offspring, and potentially leading to complications in the modern world, such as increased gestational weight and glucose intolerance. Chung *et al.*[2] showed that the nature of the colonizing microbiota affects the host's initial T cell populations, providing further evidence of host-linked co-evolution of the microbiota and immune responses. The work of Olszok *et al.*[3] was complementary to this in showing the importance of the resident microbiota in quenching the development of specific immune cells that promote systemic pro-inflammatory effects. Finally, Cho *et al.*[4] showed that early-life antibiotic exposure affects the long-term development of adipose tissue, lean muscle and bone. In total, such studies advance our views of the microbiome as an integrated metabolic space within the host at crucial times in development.

Peer Bork. I'm personally fascinated to see how broad the gut–brain–microbiota axis seems to be and how the microbiota might even affect complex human behaviour (and probably the behaviour of animals). Some have called the massive network of neurons surrounding our gut the 'second brain'; now, first indications substantiate an intensive crosstalk between microbial and human cells that goes far beyond an influence on the immune response. Although these fascinating findings let us look into the future, I currently value both solid proof-of-principle studies and statistically robust findings that prove earlier claims or hypotheses, to justify the enthusiasm of many for the emerging field of microbiomics. Elucidating the probable stratification of the human gut microbiome exemplifies the former, whereas a large-scale metagenomic association study linking the gut microbiota to type 2 diabetes is an example of the latter. Both examples belong to a growing number of findings that are paving the way to using the microbiome to improve human health in the near future, despite our still very limited understanding of microbial communities and their interactions with the human body.

Claire Fraser. The most important findings to date are: the notion that we as humans are a superorganism, with our biology determined by the genes encoded in our DNA together with the genes of our microbial partners; the extent of interindividual variability in the human microbiota in terms of taxonomic assignments (indicating that different community types are associated with health); the identification of shared sets of functions across different community types (the idea of a core microbiota); the significant impact of perturbations such as diet and antibiotics on the gut microbiota; and the concept that the "disappearing human microbiota" (as described by Martin Blaser and Stanley Falkow[5]) may be linked to the emergence of modern diseases. Taken together, these ideas suggest that it is time to take a more holistic view of health and disease.

Rob Knight. The most surprising findings, in my opinion, have been the links between the microbiome and the nervous system, including effects on neurodegenerative disease (in a mouse model) and on behaviour (in flies and mice). The transmissibility of changes in the microbiome in pregnancy from humans to mice, along with corresponding phenotypic changes, would come a close second. The value of these findings remains to be determined, however, although it would be spectacular if the microbiota were found to affect mate choice (as it does in flies) or appetite (as it does in mice). The most valuable findings to date have been links between the microbiome and drug metabolism, including use of the microbiome itself as a drug target.

Jun Wang. It has been estimated that the microorganisms in our bodies collectively add up to 100 trillion cells, ten times the number of human cells, and it has been suggested that they carry 300-fold more unique genes than are present in our own genome. Most of these microorganisms reside in the gut, have a profound influence on human physiology and nutrition, and are crucial for human life. A very surprising finding has been that disruption of the homeostasis between the microbiota and the host, known as dysbiosis, has a more important role than host genetics in the development of a range

of diseases, such as inflammatory bowel disease, obesity and type 2 diabetes. This suggests that it would be more practicable to monitor, prevent or even cure human disease by regulating the human microbiota. Thus, studying the gut microbiota will, no doubt, be crucial for the development of personalized healthcare in the future.

Most of the studies examining the role of the microbiota in human health have relied on comparing the composition of the gut microbiota in diseased and healthy individuals. What are the limitations of such studies, and what changes in study design are necessary to draw meaningful conclusions?

M.B. Clinical investigation is always challenging in a free society that needs to conduct ethical studies. Chronic illnesses are often complex; they vary in their course, subjects are often treated in many different ways, disease phenotypes are varied, compliance with medication and diets is not a given, and usually it is not possible to test causal relationships. With rare exceptions, we cannot inoculate experimental subjects with aetiological agents to fully understand causality. Despite all of these limitations, variation can be reduced by studying diseases with very clear phenotypes, and by studying them as early in their natural history as possible and before relevant therapies are given. Examining responses across an effective therapy course (and comparing with the results of ineffective therapy) can help with hypothesis testing. A longitudinal study design instead of cross-sectional studies also allows more direct testing of questions related to the pathogenetic cascade.

P.B. The field is still young, and we have only a limited knowledge of the confounding factors, the natural variation in the healthy population (and also variation over time), the impact of low-abundance species that are difficult to detect, and the required underlying community models that enable proper statistical tests to be carried out; with this limited knowledge, we indeed cannot be confident about the significance of the many observed differences between healthy and diseased individuals. Furthermore, comparing data from different studies is difficult because sample processing and analysis methodologies are still not robust. Moreover, many studies have a limited number of participants, and quality controls, such as replicates, are missing. Long-term longitudinal studies are also needed to monitor disease progression and to characterize gradients and switches in the taxonomic and functional composition of the microbiome that lead to, or might even define, the disease state. The inclusion of all of the above would significantly improve the study design. However, to keep the research affordable, one has to start simple and improve gradually.

C.F. We still don't have sufficient information about long-term variations in the healthy microbiota in response to important factors such as diet, environmental exposure and age, for example, to be able to draw robust conclusions when we observe dysbiosis associated with disease. Moreover, with most of the studies done to date, the human microbiota associated with disease is characterized only after a disease phenotype has emerged, raising the important question of what came first — a disease or a change in the microbiome. I suspect that there is no single answer to this question, but it would be very informative to be able to characterize disease-associated changes in the microbiome before and after disease onset in individuals. In addition, certain types of study (that is, intervention studies and studies across the entire gastrointestinal tract) are not feasible in humans, and additional work in various animal models should be considered as an important adjunct to the Human Microbiome Project (HMP).

R.K. These studies were valuable for establishing the concept that the microbiota could be linked to disease, but are no longer valuable except as preliminary studies showing that an effect exists, and they should not be published by themselves, or funded except as exploratory work. The main limitations are that causality cannot be known, and the differences may be due to generalized side effects of disease (for example, inflammation) rather than due to the specific condition. The gold standard is a prospective cohort study, although such studies are not always feasible. Human studies complemented by transfer of the phenotype to previously germ-free mice via the microbiota are extremely valuable for establishing causality and for follow-up studies to understand mechanisms.

J.W. Research comparing the composition of the gut microbiota in diseased and healthy individuals indicates an association between disease and the gut microbiota. The design of such projects should be cautious when it comes to the population samples studied, as well as the diet patterns of the tested individuals. In other words, the differences between the study cases and controls should not be due to different host genetic backgrounds or due to the subject's diet patterns. Besides the design, the limitation of such studies is that they attempt to prove causality based on a correlation between changes in the gut microbiota and disease. Instead, it will be crucial to carry out experiments in animals to assess the molecular mechanisms of disease, as well as to examine the function of the gut microbiota in this context.

Large projects such as the HMP and Metagenomics of the Human Intestinal Tract (MetaHIT) aimed to characterize the human microbiota and to examine its role in health and disease. In your opinion, do we need another large-scale, consortium-driven microbiome project, and if so, what would the specific aims be?

M.B. For a new research field such as the study of the human microbiome, much of the conceptual and technical infrastructure had to be built from scratch. Both the HMP and MetaHit contributed greatly to these infrastructures and have helped establish a general vocabulary, operating lexicon and tool-kit for further studies. However, we have only scratched the surface. New analytical tools are needed for measuring relevant molecules (whether they be nucleic acids, proteins or metabolites), and the data complexity requires substantial new informatic

Glossary

Framingham study
A longitudinal cardiovascular study that begun in 1948 in Framingham, Massachusetts, USA, and is still ongoing.

Longitudinal study
A study that assesses the relationship between variables over long periods of time but at regular intervals.

NHANES study
(National Health and Nutrition Examination Survey study). A set of longitudinal studies combining interviews and physical examinations that assess the health and diet of adults and children in the United States, with an aim to determine the risk factors for diseases.

Prebiotics
Substrates that are preferentially metabolized by a limited number of species and may thus be used as dietary supplements to promote targeted growth of these microorganisms.

Probiotics
Live microorganisms that confer a health benefit on the host when administered in adequate amounts.

Prospective cohort study
A longitudinal study of individuals (cohorts) who are initially assessed for their exposure to certain risk factors and then followed over time to evaluate their progression towards specific outcomes (often disease).

approaches. The greatest progress will come from analyses of disease versus control either in humans or in the animal models that show the strongest phenotypes. Strong phenotypes facilitate predictive models; in their absence, it is rough sledding. Both consortial and investigator-initiated studies are needed at this point.

P.B. Large is relative, and the cohort size depends on purpose and feasibility. For example, when we started our my.microbes community project in the fall of 2011, we were aiming at 5,000 deeply sequenced (circa 5 Gb each) metagenomic faecal samples, provided by ordinary people who were interested enough to contribute to the costs. At the time, only 124 such gut metagenomes had been published by the MetaHit consortium, and 5,000 seemed like a gigantic number. Now, several much larger studies are envisioned or have been initiated, and they are indeed needed, as many questions, even basic ones, require large cohorts and sufficient sequencing depth. Studies of the associations between the microbiota and properties of the human host are comparable to genetic linkage analyses 20 years ago, in which larger cohorts were needed in order to capture more subtle or complex associations with diseases or phenotypes; those studies also falsified a lot of the early claims in the field.

Beyond diseases, a burning question concerns the impact of diet on our individual microbiota (and how the microbiota influences our digestion), but to investigate this comprehensively and to take into account the multiple confounding factors, vast and diverse cohorts are needed. The same will be necessary to obtain an unbiased correlation between the human host and different microbial genotypes (on top of the many associations between individual human genetic variations and particular aspects of our microbiota).

C.F. I think it is worth considering longitudinal studies such as the Framingham study or the NHANES study to assess long-term changes in the human microbiota in individuals within a family structure in a given community setting. This will be useful only if extensive metadata about host genotype, diet, lifestyle, illnesses, prescription drugs, dietary supplements and so on are collected in parallel. In addition, the data to be collected about the microbiota must focus on both the structure and function of these complex communities using a variety of omics approaches in an integrated manner.

R.K. There are three leading candidates. First, a large prospective longitudinal study of children, with detailed tracking of health outcomes, would be extremely valuable for understanding when differences in the microbiota can predict differences in health. Second, a parallel to the Human Genome Diversity Project focused on characterizing the microbiota of divergent populations, including geographically and culturally isolated populations before they adopt elements of the Western lifestyle, may be crucial for understanding the suite of so-called Western diseases and for understanding the co-evolution of our microbial genomes with our host genome. Third, a large cross-sectional study of the population aimed at understanding the major sources of variability in everyone, not just in healthy people, and at providing leads about which diseases are most likely to be associated with the microbiota is really important. We are trying to bootstrap the last project using crowdfunding and crowdsourcing with the American Gut campaign.

J.W. We do need more large-scale, consortium-driven microbiome projects. For example, the projects could be, but not limited to: systematic studies of the human microbiota and their role in disease at the gene and species level; long-term prospective cohort studies to follow up a group of subjects and study the impact of the microbiota on human health; isolation and functional

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Peer Bork is a senior group leader and joint head of the Structural and Computational Biology unit at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, where he also serves as the strategic head of bioinformatics. In addition, he holds an appointment at the Max Delbrück Center for Molecular Medicine in Berlin, Germany. He received his Ph.D. in biochemistry (1990) and his habilitation in theoretical biophysics (1995). He works in various areas of computational biology and systems analysis, with a focus on function prediction, comparative analysis and data integration. He was the recipient of the prestigious Royal Society and Academie des Science Microsoft Award for the advancement of science using computational methods, and he obtained a competitive European Research Council advanced investigator grant.

Claire Fraser is Director of the Institute for Genome Sciences at the University of Maryland School of Medicine, Baltimore, USA. She has a long-standing interest in the application of large-scale genomics approaches to the study of microbial diversity and evolution. Her current research interests are focused on the structure and function of the human gut microbiome in health and disease. Previously, she was President and Director of The Institute for Genomic Research (TIGR), where she led numerous microbial genome-sequencing projects. She is the recipient of many awards and is a member of the Institute of Medicine of the US National Academy of Sciences.

Rob Knight obtained a Ph.D. in ecology and evolutionary biology from Princeton University, New Jersey, USA, in 2001 for work with Laura Landweber on the evolution of the genetic code. He then carried out postdoctoral work with Michael Yarus in the Department of Molecular, Cellular and Developmental Biology at the University of Colorado at Boulder, USA, from 2001 to 2004. He is now an associate professor in the Department of Chemistry and Biochemistry at the University of Colorado at Boulder and a Howard Hughes Medical Institute early career scientist. His current research interests are microbial ecology and evolution, the human microbiome and the evolution of functions from random sequence pools.

Jun Wang is Executive Director of the BGI (previously known as the Beijing Genomics Institute), Shenzhen, China. He was instrumental in the founding (in 1999) and growth of the BGI Bioinformatics Department, which is now widely recognized as one of world's premier research facilities committed to excellence in genome sciences. He also holds a position as Ole Rømer Professor at the University of Copenhagen, Denmark. He has been recognized with an award from the His Royal Highness Prince Foundation in Denmark, an Outstanding Science and Technology Achievement award from the Chinese Academy of Sciences, a Top 10 Scientific Achievements award in China and the prize for Important Innovation and Contribution from the Chinese Academy of Sciences. His research focuses on genomics and related bioinformatic analyses of complex diseases and agricultural crops, with the goal of developing applications using genomic information.

*Listed in alphabetical order.

annotation of the specific bacterial species; and studies examining the link between the human microbiota and both host diet and drugs, in both directions.

Q *Manipulation of the human microbiota, for example through the use of probiotics and prebiotics and through faecal transplants, has been proposed as an attractive therapeutic strategy, for instance for the treatment of inflammatory diseases such inflammatory bowel disease and of asthma. To what extent do you think this is a viable option today, and what new information is needed for the effective implementation of these therapeutic strategies?*

M.B. The human metagenome is orders of magnitude more manipulable than the human genome. This difference provides the opportunities to intercede to prevent and treat illness, if only we knew what was important! The use of faecal transplantation to treat colitis caused by *Clostridium difficile* seems to have at least some definite efficacy[6]. In a sense, it is a proof of principle that non-antibiotic biological manipulation of the microbiome can improve a serious, sometimes life-threatening disease. From detailed prospective comparisons of successful and unsuccessful transplantations, we should be able to understand the microbial predictors of altered pathophysiology. From these will come the next generation of scientifically developed probiotics and prebiotics. The important, often antibiotic-induced acute problem of *C. difficile*-mediated colitis should become tractable and could then be used as a paradigm for the discovery of microbiome-related preventives and therapies.

P.B. Some of it is reality already; according to experts, faecal microbiota transplantation (FMT) has a very high success rate in curing *C. difficile* infections. Also, for some other diseases such as inflammatory or irritable bowel diseases, there is more than hope, meaning that FMT is already therapeutically used. However, there is room for improvement in those cases — for example, by applying microbiome-based patient stratification to improve response rates to treatment and to allow more personalized FMT. For formula-based nutrition supplements, we need to know more than simply the species composition of microbial communities; we need to understand how the communities function as ecosystems. Although details about the composition of individual microbiomes might be sufficient to diagnose some extreme states, such as those observed in some diseases, and might even indicate in which direction to skew the microbiome composition to reduce imbalances, such knowledge is unlikely to reveal the ideal community needed for health. To understand what represents and contributes to a healthy state, we need to learn much more about the individuality of the microbiota at the highest resolution; that is, its composition at the strain level, its dynamics, its metabolic potential and the restrictions coming from the human host. Analogous to classical pharmacology, there will probably be only a few 'blockbuster' microorganisms that are good for everybody (but also, the dosage will be important to avoid unbalancing the microbial ecosystem) because there will probably be multiple healthy states.

C.F. The human microbiota as a target for therapeutic intervention in both health and disease is an exciting possibility. To fully consider that, we should have a strong foundation of knowledge about how the microbiota changes both structurally and functionally in response to various perturbations. Prebiotics and probiotics versus faecal transplants represent very different approaches for the manipulation of the microbiota. More rigorous scientific studies of the role of prebiotics and probiotics in health and disease are warranted, and both existing and new probiotics should be evaluated for their effects in health and disease. However, the current regulatory environment conspires against large-scale trials of prebiotics and probiotics for therapeutic purposes, and a more enlightened regulatory approach is necessary that appropriately addresses safety and efficacy without imposing unnecessary burdens on manufacturers, and that allows consumers to make informed choices.

R.K. Faecal transplants have an >90% success rate and are starting to be posited as a possible first-line therapy for *C. difficile*; given this success, it would be worth trying faecal transplants as a treatment for a wide range of other conditions, although caution must be taken, as the risk of pathogen transmission is potentially high. However, in general, the barriers to applying microbiome-based therapies have been that we don't know what 'good' looks like, we don't know what 'bad' looks like and we don't know how to get from bad to good. Surveys such as those I disparage above, in my response to the question about the limitations of comparative studies, have been very useful for understanding the difference between good and bad, but we really lack the ecological and mechanistic understanding of the parameters that control composition and change in the microbiota to make it do our bidding. Considerable additional work, both empirical and theoretical, needs to be done before we can predict which intervention is right for a given person's microbiota (and perhaps genome).

J.W. Homeostasis between the microbiota and the host is crucial to maintain human health, and disruption of this homeostasis is associated with diseases such as Crohn's disease, chronic periodontitis and obesity. I believe that new therapeutics and diagnostics which enable the manipulation of our microbiota to treat and prevent disease will be very promising in the near future. However, there are still many challenges ahead of us in the development of microbiota-targeted therapies. First, an association between changes in the microbiota composition or function and a disease is not enough to support the idea that microbiota-targeted therapies could cure the disease or relieve its symptoms; in short, association does not equal causality. Second, unlike targeting a single bacterial species, it is still very challenging to target a whole microbiota (a community of bacteria). We would also need to understand the molecular mechanism of action of the drug at the level of a protein–ligand interaction. Third, ecology studies have proved that perturbations often ripple through an ecosystem, leading to unexpected outcomes. Therefore, the variation of the gut microbiota composition that might be caused by microbiota-targeted therapies might also have different results in different individuals. Larger samples need to be examined to develop more solid therapeutic solutions.

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Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION

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