# Translating the Microbiome: What's the Target?

Two celebrated scientific movements, precision medicine and microbiome science, promise to converge on clinical medicine. Both are relevant to a diversity of clinical disciplines and capture the attention of increasingly expectant patients seeking individualized treatments based on exquisitely precise diagnostics. Clinicians responding to requests for a microbiome test often have no understanding of how such a test might be interpreted. Other than known infectious agents, what can a doctor find in a single isolated fecal microbiome analysis that is therapeutically actionable?

The most successful translation of microbiome science to clinical medicine has been in the setting of specific identifiable microbial targets, such as infection with *Helicobacter pylori* and overgrowth of *Clostridioides difficile*. Despite this, microbiome science is attracting huge commercial interest, with hundreds of start-up companies exploring microbiome-based therapies and diagnostics—already with some spectacular failures. Not until 2022 was the first microbiome-based therapeutic product approved by the US Food and Drug Administration; this is a refinement of an old treatment for recurrent *C difficile* and a reminder that microbiome science is still catching up with established clinical truths.

The most important obstacle to clinical translation of microbiome science is the unresolved question: Are microbiome alterations in human diseases causative or consequential and/or contributory to disease modification? In this Commentary, we summarize evidence favoring a consequential role for the reported microbiome alterations in many chronic human disorders and propose that the focus of clinical microbiome research should shift from blind pursuit of microbiome modulation toward identifying specific microbes and their metabolites influencing the host response and disease severity.

A significant impediment to establishing the equivalent of Koch's postulates for microbiome-related disease is the reliability of animal models for representing complex, multifactorial disease; despite this challenge, a 2020 review found that 95% of published studies using human to rodent microbiome transfer reported a transfer of pathologic symptoms.[1] This figure is implausible against a backdrop of different physiology and different native microbiomes in humans and rodents. The challenge is particularly acute for establishing models for human behavioral and cognitive functions, for which gut–brain links have been proposed.

New statistical approaches will improve the interpretation of existing cohort data[2] but cannot overcome weak or absent evidence for cause and effect. Interpreting variance of the microbiome in human disease is problematic because a normal or healthy microbiome has not been defined,[3] most of the variance have not been explained, and confounders and the clinical significance of interindividual microbiome variation are not adequately understood. Microbiomes differ with the age and lifestyle of the host and vary considerably between different ethnic groups, geographic regions, and even different individuals in the same community.[4] Moreover, most of our understanding of the human microbiome is based on studies of affluent people living in industrialized countries.[3] Not surprisingly, for most diseases where a disturbed microbiome has been implicated, no mechanisms have been conclusively identified.

Studies in human disorders need to offer more incisive evidence than the loss of microbial diversity that has been described for many disorders in which the microbiome has been implicated. Diversity analysis alone is an inadequate, nonactionable, misleading, and simplistic metric when it is agnostic of the microbial taxa that are gained or lost. Of greater concern is that few disease-specific microbial signatures have been consistently identified. Instead, commonalities or overlapping microbial changes have been found repeatedly, especially the loss of commensal species associated with healthy subjects and the outgrowth of a general subset of disease-associated commensals termed "pathobionts."[3] Duvallet et al[5] studied microbiome changes across 28 case-control gut microbiome studies spanning 10 diseases and noted that half the microbiome changes were shared across the diseases studied, and indeed there is sufficient commonality across diseases to derive a predictive index for health status based on such shared taxa[6] and to diagnose multiple diseases.[7] Many of these taxa are also those we reported as more abundant in unhealthy aging.[8] Some of the commensals repressed in multiple diseases overlap with (known or suspected) anti-inflammatory microbes first proposed as being depleted in inflammatory bowel disease, whereas pathobionts are often associated with inflammation in multiple diseases.[9] This raises the likelihood that these microbiome changes are consequential rather than causative, but it is not possible to be definitive because of the observational study design and the fact that most studies report relative abundance rather than absolute. However, some microbiome changes, such as a bloom of Proteobacteria commonly found in inflammatory bowel disease and other disorders, are indeed consequential and predictable because of increases in mucosal oxygen tension in the presence of inflammation.[10] Moreover, comparative analyses of paired biopsies from inflamed and noninflamed colonic mucosa and longitudinal studies during relapse and remission provided no circumstantial support for disease causality of the microbiome.[11] In other chronic conditions where the microbiome has been implicated, the microbial disturbances have since been shown at least in part to be due to drug treatment, as in type 2 diabetes,[12] or, in the case of autism, to result from dietary preferences.[13]

Another feature confounding the interpretation of apparent microbiome abnormalities in several human diseases is the translocation of oral microbes to the distal gut. This is particularly evident in the inflammatory bowel diseases Crohn's disease and ulcerative colitis and in sporadic colorectal cancer and liver disease.[14] Oral microbes are detected in the gut more frequently in people with gastrointestinal disease and cancer than in healthy subjects. As early as 2016, Pasolli et al[15] (including one of us, Nicola Segata) noted from a study of over 2000 gut metagenomes that the presence of some oral microbes was a general feature of diseases involving dysbiosis including liver cirrhosis, where *Veillonella* spp, *Streptococcus* spp, and *Haemophilus parainfluenzae* were signature taxa. In addition, many of the disease-associated microbes identified by Duvallet et al,[5] such as *Porphyromonas* and *Fusobacterium*, were likely of oral origin. Oral microbes, especially *Fusobacterium nucleatum*, were not only detected in fecal samples in large colon cancer meta-analyses[16] but are consistently found in colon cancer biopsies, often as polymicrobial biofilm-forming communities. Oral microbes are more abundant in the fecal microbiota of patients with ulcerative colitis than healthy control subjects, and ectopic integration of *Klebsiella* strains from patients with inflammatory bowel disease drove gut inflammation in a gnotobiotic model.[17] However, although colonization of the gut by oral microbes was confirmed for patients with colorectal cancer and rheumatoid arthritis, it also occurred at high frequency in healthy control subjects.[18] Of note, oral microbes are highly transmissible between individuals sharing the same household, with an almost 40% median strain-sharing rate between adult partners living together for several years against close to a rate of 0% between unrelated individuals.[19] This transmission rate is, as expected, higher than that of the gut microbiomes and could mean that the likelihood of acquiring oral microbes with a role in gut inflammation is partially dependent on social and direct interactions.

Increasing availability of large metagenomic cohorts is further fueling the possibility of inferring reproducible links between the presence of oral microbes in the gut and host conditions. In an analysis of 30 metagenomic datasets (4646 total samples) spanning 15 distinct host diseases, an increased abundance of oral microbes in the gut was observed in all but 1 dataset and proved significant in half of them with a very significant overall meta-analysis association (Manghi et al, unpublished data). With respect to aging, in our 2020 study of 2500 subjects,[8] approximately half of the taxa associated with dysbiosis-type diseases in young and middle-aged subjects were classifiable as oral microbes, but only 1 of 9 taxa that were more abundant in older subjects with disease, namely *Streptococcus parasanguinis*, was an oral taxon. In a later meta-analysis,[20] we identified 19 taxa associated with unhealthy aging, 6 of which were clearly oral microbes (*Streptococcus* sp, *Actinomyces* sp, *Desulfovibrio*, *Campylobacter*, *Atopobiaceae*, and *Veillonella*). In our most recent study of 21,000 microbiome datasets,[21] a newly defined index, Kendall Uniqueness, showed the strongest association (negatively, for this metric) with retention of health during aging. Twelve of 22 taxa that associated positively with Kendall Uniqueness (indicating unhealthy aging) were oral microbes, with the remaining taxa being clearly intestinal microbes. Interestingly, 8 of these oral microbes formed a separate co-abundance network (see Figure 5 in Ghosh et al[21]), possibly reflecting the tendency of oral microbes to form mixed biofilms. Although there is a selection bias in published studies because young adults that are sick are less likely to survive and be surveyed as older subjects, we conclude that introgression of oral microbes is indicative of, but not sufficient for, loss of health in aging.

There is a robust case for using microbiome analysis for disease detection or diagnosis such as for colon cancer,[22] potentially autism[23] recognizing dietary influences discussed above,[13] and other diseases.[7] However, the quest for a causal link with the microbiome for many chronic human disorders is undermined by the common occurrence of nonspecific, overlapping, and expected microbiome disturbances. Targeting secondary microbiome changes is unlikely to yield sustained disease remission, but significant therapeutic improvement can still be achieved by addressing specific disturbances of the microbiota that exacerbate disease severity. An insightful example of this strategy is the recent clarification of the role of the microbiome in the inherited human disorder, familial dysautonomia. This monogenic disorder is characterized by progressive degeneration of the peripheral and central nervous systems with deficits of innervation in many organs, including the gastrointestinal tract. This leads to disturbances of the microbiome and metabolome, which likely exacerbate the neurodegeneration.[24] Similarly, with more common and more complex conditions such as chronic kidney disease, there is a role for the gut microbiota modulating disease severity, regardless of the cause.[25] Improved responses to existing treatments and dietary regimes may also be achievable with microbiome modulation as shown by encouraging evidence linking the microbiome with responsiveness to cancer immunotherapy. It is also possible that part of the missing causal links can be unraveled by multiomic studies or by mechanistic insights that have to be explored at the level of profiling the functional and metabolic capabilities of individual strains, but these tasks are limited by challenges related to high-dimensional statistics and stubbornly prohibitive costs of high-depth metagenome sequencing, especially for research groups in low- or middle-income countries. Thus, overall, the promise of microbiome-based therapeutics is alive, but more likely to be fulfilled with identification of specific disease-modifying microbial metabolites.

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**Received February 27, 2023. Accepted April 16, 2023.**

**Conflicts of interest**
This author discloses the following: Paul W. O'Toole is a research proposal reviewer and advisor for The Weston Family Foundation and the US Highbush Blueberry Council. The remaining authors disclose no conflicts.

**Funding**
Paul W. O'Toole and Fergus Shanahan are supported in part by awards from the Science Foundation Ireland to APC Microbiome Ireland, the Department of Agriculture, Food & Marine (government of Ireland), the Horizons Europe program MASTER-818368, the European Commission under the European Union's Horizon 2020 Research and Innovation Programme (grant agreement SC1-HCO-17-2020 No. 964590-IHMCSA), and philanthropic donation. Tarini Shankar Ghosh is supported by the Department of Biotechnology, Ministry of Science & Technology, Government of India for the Ramalingaswami Re-entry Fellowship (BT/HRD/35/02/2006) and IIIT-Delhi for a Research Initiation Grant. Sourav Goswami is supported by an Institute Fellowship at IIIT-Delhi. Nicola Segata is supported by the European Research Council (ERC-STG project MetaPG-716575 and ERC-COG project microTOUCH-101045015), the European H2020 and Horizon Europe programs (ONCOBIOME-825410, MASTER-818368, CODIET-101084642, DOMINO-101060218), the National Cancer Institute of the National Institutes of Health (1U01CA230551), and the Premio Internazionale Lombardia e Ricerca 2019.

**Data Availability**
Additional supportive references for statements in this Commentary are available from authors upon request.

**Most current article**


**0016-5085**
**https://doi.org/10.1053/j.gastro.2023.04.008**