From trash to gold: gastrointestinal microbiome research in patients with functional gastrointestinal disorders

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Archaeological research yields valuable insights of past communities or civilizations by sifting through the rubbish dumps of ancient dwellings. Microbiome research targeting the gastrointestinal (GI) microbiota communities traditionally uses a similar approach. Feces, the end product of the digestive process that is excreted, are carefully analyzed using very sophisticated methodologies as if feces would hold the treasure trove filled with gold or providing the key for our understanding of the interactions of communities of microbiota with the human body and its relevance for specific diseases.

Microbiome research has taken off with the development and widespread availability of culture-independent high throughput sequencing capabilities and there are high hopes these approaches will enable medicine to answer many burning questions. Indeed, as the technology advances and provides greater scale in time-effective and cost-effective ways, the fields of molecular epidemiology and molecular microbiology further coalesce to reveal associations between the GI microbiome and immune functions, biomolecular activities, or pathogen exclusion, as well as links between the microbiome and diseases such as inflammatory bowel disease,[1] autism,[2] cancer,[3] and even so-called functional GI disorders.[4]

The rise of human microbiome research over the last three decades coincides with the elucidation of many pathophysiologic concepts for functional gastrointestinal disorders (FGID) including functional dyspepsia (FD) that overlap disordered gastric emptying, impaired funding relaxation, heightened visceral sensory function, psychological factors (or brain-gut factors) and minimal mucosal or systemic inflammation.[5] Efforts to elucidate these disease mechanisms were driven by the pressing need to develop therapies for these patients and the emerging pathophysiologic mechanisms mentioned above virtually always initially offering hope that they would allow targeting of underlying disease mechanisms and ultimately provide cure for patients with FGID. Sadly, these expectations are, at best, only partially met. As such, while some of the physiologic insights may be of value to better understand the multifaceted pathophysiology of FGID, this knowledge has not been sufficient for the development of new treatments that provide a 'cure' for the majority of patients with FGID.

Against this background, perhaps it is not surprising that there has been a surge of microbiome studies aiming to establish whether and how changes of the GI microbiome are related to specific FGIDs. Most of these studies have focused on the stool microbiome, in no small part because of the logistics of 'collecting trash to seek treasures'. However, the decision to focus on the faeces, while greatly informative, may not always be the most appropriate source of information. There is already good evidence that the stool microbiome is substantially influenced by transient environmental factors such as diet[6] and/or GI transit, including greater methane positivity and resident archaea in subjects with slower transit times, irrespective of health status.[7] Indeed, strong associations have been reported between stool consistency and species richness, 'enterotypes', and community composition.[8] Moreover, while the advent of culture-independent measures has expanded our awareness of the taxonomic and functional diversity inherent to the gut microbiome in compositional terms, the quantitative assessment of total microbial abundance in stool biomass has waned, until recently.[9] Thus, some stool parameters frequently measured and reported from culture-independent approaches may provide an 'objective measure' of laxation frequency, and/or diet composition, rather than the functional insights into whether and how specific disease conditions are linked to the stool microbiome.

Since the findings reported by Gevers *et al* establishing the stool and mucosa-associated microbiome (MAM) are different in pediatric subjects with new-onset inflammatory bowel disease,[10] there has been a growing interest to better characterize this component of the gut microbiome, including the upper segments of the GI tract.[11] Here, Cervantes *et al*[12] have used an integrated combination of culture-dependent and independent techniques to

examine microbiome composition in saliva, and the gastric and duodenal mucosa of patients with FD and controls (with achalasia). They note that in patients with FD there are changes in the taxonomic composition of the saliva, gastric, and duodenal microbiomes and in particular, the relative abundance of *Veillonella* spp in saliva increases, and there is a shift to a greater representation of oral bacteria (although not *Veillonella* spp) in the gastric region of patients with FD. Furthermore, metabolic function prediction identified greater anaerobic metabolism in the stomach microbial community of patients with dyspepsia. Interestingly, the differences in the duodenal MAM between patients with FD and controls appear more diverse, but the authors note the 'oral shift' is not as pronounced at this site. Their co-abundance analysis revealed that members of the *Rothia* genus act as a key hub in the duodenum MAM, a genus that is significantly (positively) correlated with the relative abundances of *Haemophilus, Clostridium, Actinobacillus,* and several other Gram-positive bacterial genera, and negatively correlated with the genus *Lactobacillus*. While the nested PCR approach employed by the authors to examine these communities may affect the coverage and accuracy of relative abundance measures,[13] their overall findings do complement other work that points towards small intestinal dysbiosis in at least a subgroup of patients with FD[14] and irritable bowel syndrome.[4]

One might consider the examination of the stool microbiome to possess an archaeological context of gut microbial function, not unlike expeditions that aim to understand complex ancient civilisations by focussing on their ancient rubbish deposits. In contrast, sampling the MAM at various segments of the GI tract offers a more 'real-time' and site-specific assessment of the microbiota, their interactions with the mucosal immune system, and thereby their direct linkages with host phenotype (symptoms), providing additional insights into the pathophysiology and potential approaches towards new therapeutic interventions. However, the sampling and characterization of the MAM is not without challenges of its own. For instance, and compared with stool, the size of MAM populations is much smaller. This limitation is overcome via the use of bacteria-specific primer sets for community profiling, and there is a growing number of studies that confirm the MAM of various segments of the GI tract (and regions within large intestine) are taxonomically distinct.[15] Here, Cervantes, McCallum *et al* have used sterile brushes to obtain superficial samples, which can provide a more extensive 'sampling' of the mucosal surface, and while there are inherent advantages of using such an approach there can be compositional differences in the MAM profiles from samples collected using standard or aseptic (sheathed) biopsy forceps, and superficial brushing.[16] Furthermore, it is not only the relative abundance of different genera but the overall bacterial load that might be of relevance for disease processes in FD.[17] Although PCR-based approaches to measure bacterial load were not reported here, it is interesting to note that the culture-based methods used suggest increased total (and particularly anaerobe) bacterial counts from superficial brushings of subjects with achalasia compared with the dyspeptic cohort. These interesting findings complement recent data linking increased bacterial load on duodenal tissue with the impairment of quality of life in patients with FD.[18] Of further interest is the biodiversity of bacteria recovered using culture-based methods in this study: although key bacterial taxa (eg, *Rothia, Veillonella*) were not apparently recovered, isolates representing 'missing' or 'low abundance' taxa in the culture-independent duodenal MAM profiles are recovered.

In summary, the integrated utilization of both culture-dependent and culture-independent approaches used here by Cervantes, McCallum *et al* adds value to characterizing the MAM of the upper GI tract, and its relevance to dysregulated gut function. Going forward, these approaches and others will enable better integration of the emerging knowledge of the 'gut microbiome' colonizing different segments of the GI tract with other established or potential pathophysiologic mechanisms, including alterations of sensory/motor function or the previously described inflammatory processes and the link with psychological stressors.[19]

These are complex tasks. However in contrast to archaeologists, who often rely on excavations and sifting through ancient rubbish dumps in the hope of discovering hints of the form and function of ancient communities, gastroenterologists and microbiologists are now well positioned to have a laser-sharp focus on microbe-host interactions and systematically explore both mucosal and luminal microbial communities, their links with established disease mechanisms such as immune function,[20] or the role of the microbiome as an important factor in modulating gut-brain signaling.[21] These approaches, when used as part of clinical studies like those of Cervantes, McCallum, *et al*, are likely to pave a straighter path to find 'gold' or the holy grail: knowledge that that can be translated into diagnostic and therapeutic benefits for large patient populations with underlying GI diseases and disorders.

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