OPINION

Gut microbiome as a clinical tool in gastrointestinal disease management: are we there yet?

Eamonn M. M. Quigley

Abstract | Spurred on by ever-evolving developments in analytical methodology, the microbiome, and the gut microbiome in particular, has become the hot topic in biomedical research. Ingenious experiments in animal models have revealed the extent to which the gut microbiota sustains health and how its disruption might contribute to disease pathogenesis. Not surprisingly, associations between the microbiota and disease states in humans have been the subject of considerable interest and many links proposed. However, with rare exceptions, the incrimination of an altered microbiota in disease pathogenesis seems premature at this time given our incomplete understanding of the composition of the gut microbiota in health and the effect of many confounding factors in the interpretation of supposedly abnormal microbial signatures. Future studies must account for these variables and the bidirectionality of host–microorganism interactions in health and disease. In this Perspectives, the status of microbiota signatures in the clinical arena (for facilitating diagnosis or refining prognosis) will be critically assessed and guidance toward future progress provided.

Few areas of biomedical science have witnessed such a rapid explosion in knowledge as that relating to the gut microbiome — the microbiome revolution[1]. Over the past two decades, our eyes have been opened to the various parts that our commensal bacterial populations play in keeping us healthy; not surprisingly, clinical and laboratory researchers have rushed to examine associations between the gut microbiome and various disease states. Initially, and for obvious reasons, the focus was on gastrointestinal diseases whereby examples of the effect of a disturbed gut microbiota were already present: enteric infections, *Helicobacter pylori*-related diseases and antibiotic-associated diarrhoea[2]. Over the past decade, and facilitated by rapid and ever-evolving progress in techniques that enable us to enumerate intestinal bacteria, their genes and metabolic products[3], we have witnessed claims for associations between the gut microbiota and a broad spectrum of neuropsychiatric, immunological and allergic disorders[4]. An altered microbiota has, for example, been implicated in a host of apparently diverse disorders ranging from Parkinson disease[5] and autism[6] to diabetes[7], asthma[8] and coeliac disease[9].

In a very short space of time, therefore, microbiome research has moved from the laboratory into the realms of clinical practice, for which its potential in facilitating diagnosis, predicting prognosis and guiding treatment has generated considerable interest among investigators and the biomedical industry alike. Three assumptions underlie a belief in the clinical applicability of microbiome research: first, that we know what is normal; second, that we can accurately and reproducibly define what is abnormal; and, third, and perhaps most important, that we can establish a biologically plausible and clinically meaningful relationship between a certain microbiota or microbiome profile and a given disease state.

For the purposes of this Perspectives, I will use the following definitions for clarification. The microbiota refers to the assemblage of microorganisms (and not just bacteria) present in a defined environment; by contrast, the microbiome comprises the full complement of microorganisms (bacteria, viruses, fungi and protozoa), their genes and genomes in a given locus (for example, the gut). It must be conceded that these terms are often used interchangeably in the literature to refer to microbial communities.

What is normal?

Despite advances in analytical techniques and their interpretation, our understanding of the composition and function of all of the bacterial populations, not to mention other microorganisms, such as viruses and protozoa, that inhabit various parts of the gastrointestinal tract remains incomplete[3]. Even the oft-quoted assumption of a 10:1 ratio between bacterial and human cells has been questioned[10]. Although much has been learned of the contributions of the microbiota to sustaining health[11], this progress does not mean that we can accurately define normality. Although fairly large population studies (ranging from the low hundreds to over one thousand) have demonstrated some commonality between healthy individuals at genus level, interindividual variation remains the order of the day at the level of species and strain[12–15]. As the factors that contribute to that variability are identified, one can begin to appreciate the extent to which factors — such as age[16,17], birth mode[18], breast-feeding or formula-feeding[19], diet[20], geography[18], exercise[21], other lifestyle factors, such as alcohol consumption[22], and exposure to antibiotics[23] — can affect any definition of 'normal' (FIG. 1). Diet might well be the foremost confounder of many human microbiome studies to date. Not only does the overall nature of a particular dietary pattern (for example, vegan versus vegetarian versus carnivore, or highly processed Western diet versus rural African diet) influence the microbiota but the relative amounts of specific components (carbohydrate, protein, fat, fibre) are also important[24]. Dietary habit

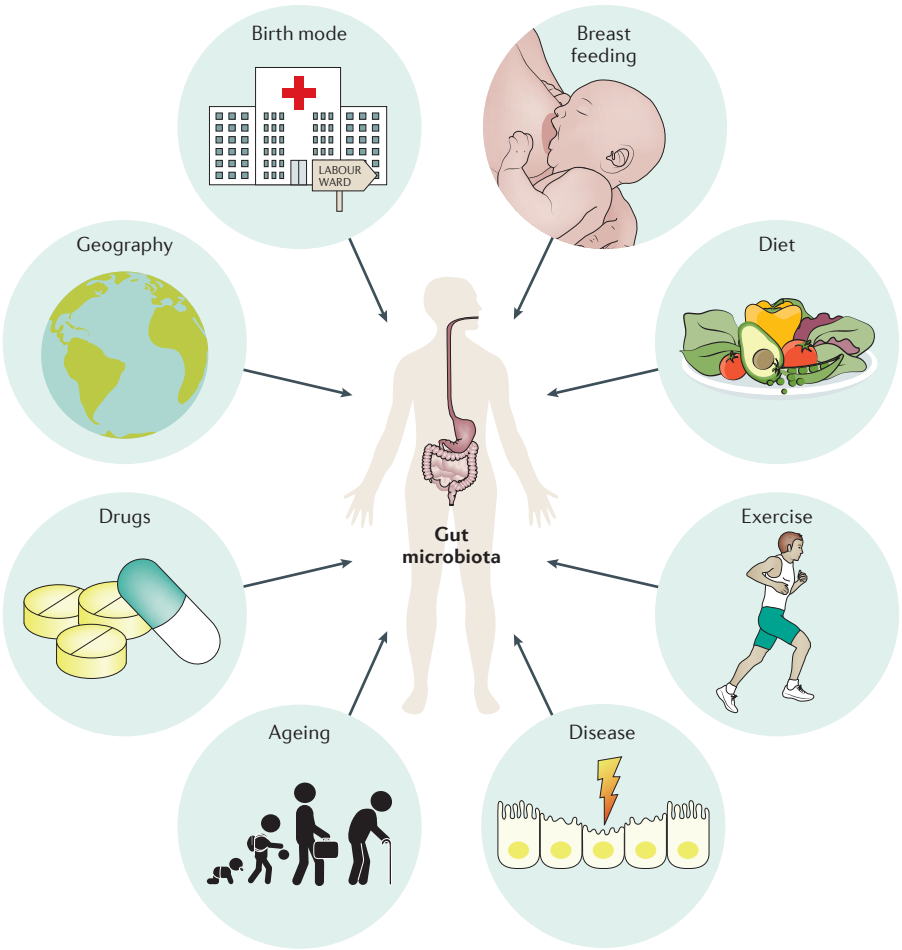

Figure 1 | **Factors that can influence the composition and function of the human gut microbiota.** Numerous factors have an influence on the gut microbiota. Clockwise from top left: methods of delivery at childbirth; whether breast or bottle fed; diet; exercise and other personal habits; presence of disease (e.g. intestinal inflammation); ageing; medications (especially antibiotics but also acid suppressants and metformin); geography.

will shape the microbiota over the long term[20,24–27], but short-term changes in diet, if sufficiently dramatic, as might occur in an individual with a gastrointestinal illness, can also fashion changes in bacterial populations[28–30]. The effect of breast milk, mediated by its own bacterial population as well as its prebiotic oligosaccharides, on the microbiota of the infant and child provides another vivid example of a powerful environmental factor[19,31,32]. Other therapeutic agents might also impose changes; antibiotics are obvious culprits and the effect of therapeutic doses of various antibiotics has been well described[33]. Less-well-documented, but potentially much more widespread in terms of their influence, are the changes in the gut microbiota described in animal models that mimic the antibiotic dosages that we obtain in our food through their use in animal husbandry[34]. As the gut microbiota changes related to other commonly used pharmaceuticals are increasingly described, we must be alert to the unexpected[35,36].

Evidence is accumulating to indicate that modifications to the gut microbiota in infancy and early childhood might be especially critical to the development of disease later in life[34,37]; efforts to ameliorate disease through the modulation of the microbiota later in life might, therefore, be doomed to failure. The final confounder and the rock on which many association studies can perish is the possibility that changes observed in the gut microbiota might be consequent upon, rather than causative of, the disease process under study. Firstly, the gut microbiota are influenced by the host genome[38] and those very same genetic traits that predispose to a given disease might *pari passu* produce a certain microbial signature. Secondly, inflammation per se, through the effects of inflammatory mediators, can disrupt bacterial populations[39] and one must also be ever aware of the fact that the microbiota–gut–brain axis, so topical at the moment, is bidirectional, as exemplified by the effects of stress on gut physiology, immune function and gut microbiota composition[40] (FIG. 2).

Microbiota in gastrointestinal disease

Understanding the context. Experiments involving a variety of *in vitro*, *in vivo* and *ex vivo* models have explored the role of the microbiome in homeostasis in health, and in the pathophysiology of gut disorders such as IBD and IBS[41–46]. At a fundamental level, many of these disorders seem to involve variable interactions between a normal or disturbed gut microbiota, microbial metabolic products, the host genome (regulating such factors as the immune response), the gut barrier (in its broadest sense)[47], the host immune response, host physiology and not forgetting interactions with dietary and other microenvironmental and macroenvironmental factors. Given that many of these interactions are bidirectional, one can readily appreciate the challenge the investigator faces in attempting to isolate the role of the gut microbiota in a given disease state.

Some progress has been made. The effects of a gross disturbance of the gut microbiota is most readily appreciated in the context of broad-spectrum antimicrobial agents and the protective role of an intact commensal bacterial community, which is vividly illustrated by the development of *Clostridium difficile* infection when the former is suppressed by antibiotic therapy[48]. A microbial signature that predisposes the individual to this potentially life-threatening infection has been described[49] and faecal microbial transplantation (FMT) has been shown to restore resistance to *C. difficile* infection *in vivo*[50].

Interactions between bacterial pathogenicity factors, the host genome and, in turn, the host immune response have been shown to have a central and interlinked role in determining the disease phenotype that emerges from infection with *Helicobacter pylori*[51]. Though the resultant phenotype is more heterogeneous, a convergence of bacterial and host immune responses is also suggested as being central to the pathogenesis of IBD, as illustrated by the prevalence of polymorphisms in genes (such as *NOD2*/*CARD15*) involved in the host response to bacteria among the multitude of genes that have been linked to both Crohn's disease and ulcerative colitis[52].

Along similar lines, single nucleotide polymorphisms have been identified in genes coding for Toll-like receptor 9, IL-6 and E-Cadherin (*CDH1*) in one subtype of IBS, post-infectious IBS, suggesting the involvement of a dysregulated immune response and impaired gut barrier function in this disorder as well[53].

In an overly simplistic concept, impaired gut barrier function has been frequently incriminated in the pathogenesis of microbiota-induced (or microbiota-related) gastrointestinal and systemic disorders. According to this model (FIG. 3), a so-called leaky gut permits the translocation of bacteria or bacterial products (such as lipopolysaccharide from Gram-negative bacteria) across the damaged epithelium, where it accesses the portal or even systemic circulations, ultimately contributing to systemic sepsis and/or immune responses[54]. Several problems remain with this hypothesis, attractive though it might be. First, measures of translocation have proven unreliable and variably reproducible in humans, in contrast to animal models[55]. Second, tests of intestinal permeability in humans, such as estimations of the lactulose:mannitol ratio, the relative absorption of polyethylene glycols of variable molecular weights or Chromium-51 EDTA clearance[56], typically involve methodologies that assess the integrity of the paracellular pathway, a pathway involved in the passage of ions and water and scarcely able to transport the large molecules of bacterial products, let alone whole bacteria (pore size of 7.5 Å versus dimensions of *E. coli* of 0.5 by 2 μm)[57,58]. This is not to say that the detection of paracellular leakiness might not serve as an indirect indicator of an insult to the epithelium, which could also injure transcellular and other pathways and result in the translocation of bacteria and/or their products. Finally, other components of gut defense, such as a gut–vascular barrier, might be central to the systemic dissemination of enteric bacteria[59].

Certain bacterial metabolic products have critical roles in the pathogenesis of symptoms and even in the aetiology of gut and systemic diseases. Bile acids enjoy a complex and bidirectional relationship with the gut microbiota. On the one hand, bile acids exert bacteriostatic effects that certain bacterial species, such as lactobacilli and bifidobacteria[60], learn to evade through the possession of the enzyme, bile salt hydrolase. On the other hand, bacterial metabolism of primary bile acids generates products that might exert, through their ever-expanding repertoire of regulatory functions, effects on host metabolism and immune responses[61], as well as on colonic motility and secretion. For example, chenodeoxycholic acid has been shown to accelerate colonic transit[62], taurodeoxycholate to modulate apical chloride–hydroxide exchange activity in intestinal epithelia[63] and, acting as the endogenous agonists of the plasma membrane bound G-protein receptor TGR5, bile acids such as cholic, chenodeoxycholic and deoxycholic acid promote glucagon-like peptide 1 expression, thereby augmenting insulin secretion and type 2 iodothyronine deiodinase (a major thermogenic protein) secretion[64]. Activation of TGR5, through effects on the expression of mediators released in response to activation of the NF-κB pathway and suppression of Toll-like receptor 4 activated pathways, can exert anti-inflammatory effects[64]. Furthermore, short-chain fatty acids (SCFAs) are an important product of bacterial metabolism of undigested carbohydrates[65]. Long recognized as critical fuels for the colonic epithelium, other effects of SCFAs, such as immune modulation and neuroendocrine signalling, are increasingly recognized[66].

Interpreting the studies. It should be evident from the aforementioned overview of pathogenetic and pathophysiological factors related to the gut microbiota that a multitude of often interacting factors might be at play in a given gastrointestinal disease and that the definition of their relative importance, although feasible in animal models, could prove very challenging or even elusive in humans. Defining what is there using high-throughput sequencing could be a good first step, but will merely document association and certainly not prove causation. A more complete delineation of a pathogenetic role of a given microbial signature might be intimated from metagenomics and supported by metabolomics. A greater emphasis on the functions of components of the microbiota, and not just a description of its component parts, must be a critical feature of future studies of the microbiota in health and disease (BOX 1). The interrogation of the bacterial metagenome using shotgun sequencing, as reviewed by Nayfach and Pollard[67], has the potential to identify bacterial metabolic pathways, infer microbial interactions and identify microbial metabolites that affect host biology. Metabolomics employing

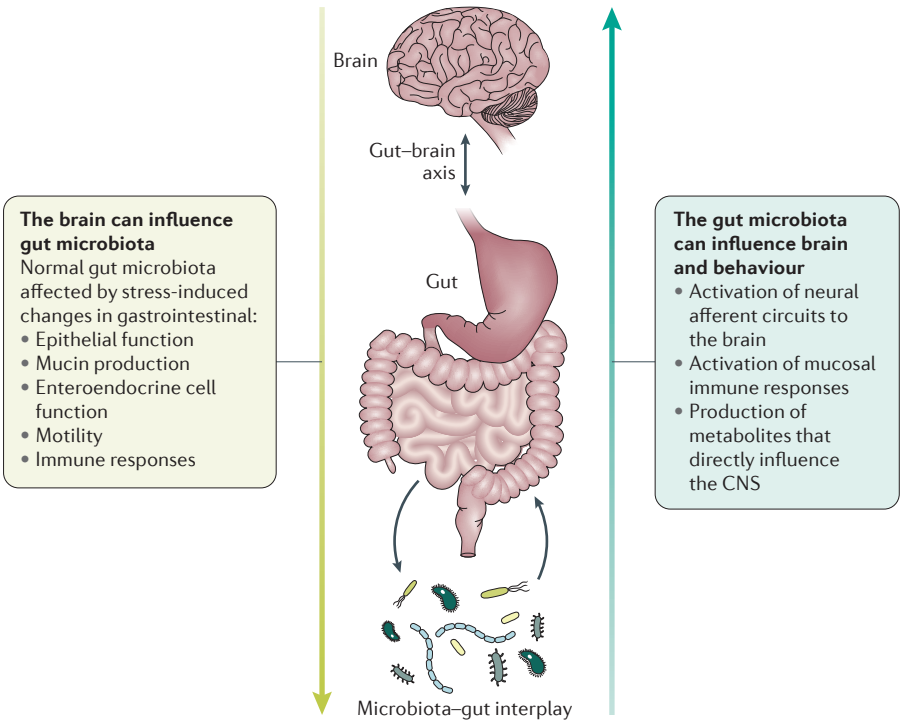

Figure 2 | **The microbiome–gut–brain axis.** A bidirectional interaction occurs between gut microbiota, the gut (including its immune and neural networks, as well as the gut barrier) and the brain. CNS, central nervous system. Modified, with permission, from Elsevier © Collins, S. M. & Bercik P. *Gastroenterology* **136**, 2003–2014 (2009).

techniques such as mass spectrometry and nuclear magnetic resonance spectroscopy can identify molecules produced by the gut microbiota and help to define metabolic pathways[68]. Other '–omics' approaches, such as metatranscriptomics and metaproteomics, have the potential to further reveal the functions of the gut microbiome. The potential of detailed interrogation of bacterial genomes coupled with the manipulation of genes of interest is exemplified by the latest studies of a particular strain of *Bifidobacterium longum* spp.[69,70]. By identifying the gene cluster responsible for the elaboration of the exopolysaccharide (EPS) coat that is so prominent around this bacterium[56] and then developing a mutant devoid of these genes, the investigators were able to define the critical part that EPS played in the immune-modulating functions of this bacterium[70]. Studies on the role of the gut microbiota in hepatic encephalopathy (perhaps the original microbiome–gut axis disorder)[71] illustrate the shortcomings of approaches limited to high-throughput sequencing alone. In an investigation of the pathogenesis of the beneficial effects of the poorly absorbed antibiotic rifaximin in hepatic encephalopathy, it was found that the amelioration of encephalopathy by this antibiotic owed more to shifts in bacterial metabolism rather than changes in the actual composition of the gut microbiota[72]. The importance of bacterial culture, not merely in the definition of viability, but also as an adjunct to genomic approaches in the identification and characterization of bacterial species, also deserves emphasis[73].

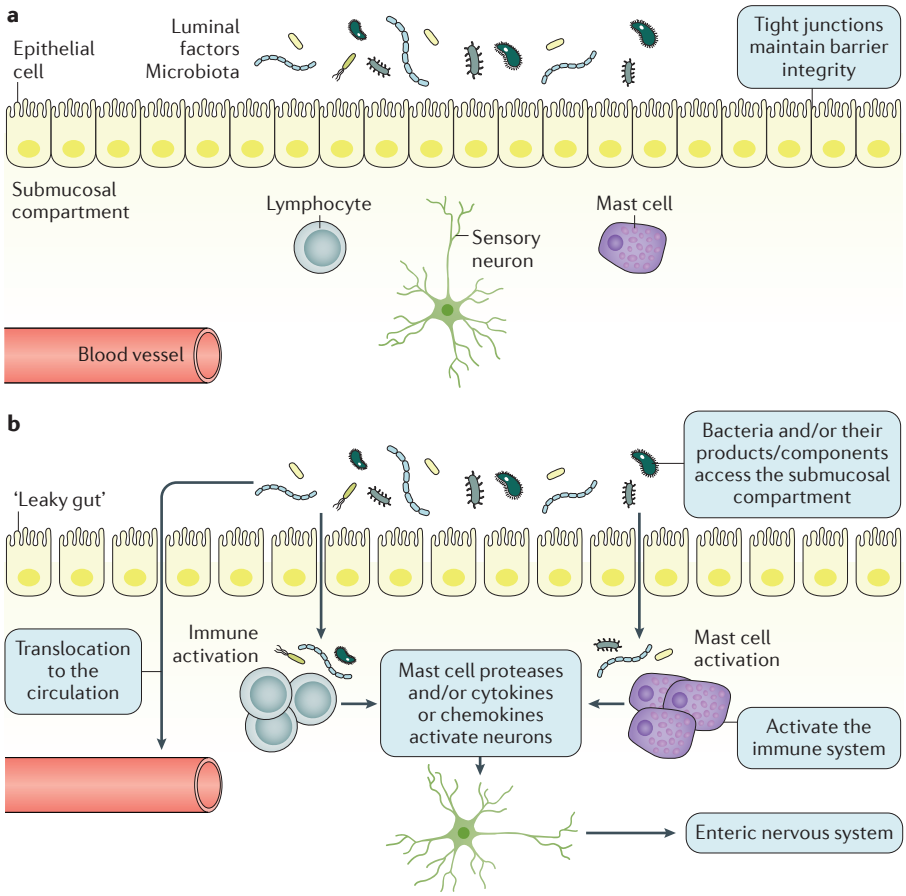

Figure 3 | **The 'leaky gut' hypothesis. a** | The normal intestine — an intact barrier, including tight junctions — prevents translocation of bacteria and/or bacterial components or products into the submucosal compartment. **b** | The 'leaky gut': disruption of tight junction integrity permits bacteria (from a normal or altered gut microbiota) to access the submucosa, where they activate mast cells and lymphocytes that release products such as mast cell proteases and cytokines and chemokines, which lead to inflammation and activation of sensory neurons. Access is also provided to the vasculature and thereby to the portal circulation, the liver and potentially the systemic circulation. As discussed in the main text, this hypothesis is an oversimplification of interactions between the microbiota and the gut barrier, and many of the aforementioned steps have not been demonstrated in humans.

Other challenges confront the clinical researcher. The effect of interactions between the microbiota with components of the diet and its metabolic products, already emphasized earlier, must be remembered in translational as well as clinical research. For example, the multiplicity of diets used by patients with IBS, from high-fibre diets to those that are gluten-free or low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (low FODMAP diets), each of which can affect the microbiome[28,30,74], should be accounted for in studies of the microbiome in this disorder.

Sampling, as well as the handling, storage and analysis of biological samples, present their own hurdles. For obvious reasons of convenience, most human studies of the gut microbiome have been based on the analysis of faecal samples. This approach ignores the tremendous variations in bacterial density and populations along the length of the gastrointestinal tract; a microbiome-based disease that primarily involves the small intestine is unlikely to be reflected in a faecal sample; changes in small intestinal populations being undetectable in the midst of the far greater bacterial populations that inhabit the colon. Furthermore, it is also clear that, at any point along the gut, differences are also evident between bacterial populations resident in the lumen and those adherent to the mucosal surface[75]. These mucosa-associated bacterial species and strains will not be accurately represented in faecal samples; a major limitation of this approach. It stands to reason that bacterial species resident at the mucosal surface, or within the mucus layer, are those most likely to participate in interactions with the host immune system and the gut barrier, whereas those that populate the lumen might be more relevant to metabolic interactions with food or the products of digestion. Evidence for clear differences between these populations in both health and disease states already exists[76–78]. Codling and colleagues[76], for example, noted far less variability in bacterial signatures from mucosal than faecal samples obtained from the same subjects. To promote economies of scale, samples are typically frozen and then analysed in batches; precisely how the sample is handled and stored and the methodology employed to analyse it will also influence results[3].

Longitudinal studies with sampling of the gut microbiota at multiple time points (a rarity in human studies) that track for disease activity and/or symptom intensity

Box 1 | **Establishing a causative role of the microbiome in human disease**

Approaches that will support causation rather than mere association:

- Study a homogenous phenotype
- Control for diet and other external factors, such as alcohol consumption, that can influence the gut microbiota
- Standardize sampling, storage and analytical techniques
- Perform longitudinal rather than single-point-in-time studies, which can track relationships between symptom and/or disease fluctuations and microbiota changes
- Sample the microbiota at their likely site of action (e.g. mucosal sampling, sampling from stomach, small intestine or colon)
- Define likely bacterial functions and products via metagenomics and metabolomics, for example
- Observe symptomatic improvement or even cure with a therapy directed specifically at the microbiota

will also assist in differentiating signals that are state from those that are trait. Ultimately, a symptomatic response or cure to an intervention directed at the microbiome should clinch its role in a given disorder; to date, *Clostridium difficile*-related disease alone fulfils these criteria[79].

With regard to the implication of the gut microbiota in human disease, proof of principle or concept can be provided by ingenious experiments using mice (either germ-free or microbiota-depleted) colonized (so-called humanized) with microbiota harvested from healthy individuals or those with disease. This approach has, for example, provided valuable insights into the short-term and long-term effects of dietary changes on the gut microbiota[80], revealed interactions between diet, the gut microbiota and gut motility[81], and supported a role for the microbiota in disease states, such as IBD[82], depression[83] or Parkinson disease[84]. Animal models can also be manipulated to define bacterial metabolic pathways relevant to human disease, such as those relevant to hepatic encephalopathy[85].

Conclusions

The microbiome revolution is certainly upon us and our basic science colleagues have thrown down the gauntlet through their elegant description of the complex and extensive roles of the microbiome in homeostasis as well as in the pathophysiology of disease in animal models. Meanwhile, the availability of high-throughput sequencing techniques has spawned a profusion of studies of the gut microbiota in almost every known gastrointestinal, liver and pancreaticobiliary disease. Results to date have been, at best, confusing and, at worst, conflicting but this aspect has not restrained an unwarranted haste to incriminate 'abnormal' bacterial signatures in many of these diseases.

However, a clear picture of the role of the gut microbiota in common gastrointestinal diseases has yet to emerge and has been hampered by a failure to account for confounding factors or to optimize sampling methods. Aware of these limitations and armed with an armamentarium of diverse microbiological tools we are now in a position to perform appropriately powered, longitudinal studies of well-phenotyped populations using a standardized methodology[86], which has real potential to uncover the role(s) of our bacterial fellow travellers in gastrointestinal disorders. Such studies are a necessary prelude to the development of novel diagnostic and therapeutic interventions. Until they have been completed, we cannot and should not offer microbiota analysis as a diagnostic or prognostic tool in routine clinical practice.

Eamonn M. M. Quigley is at the David M and Lynda K Underwood Center for Digestive Disorders, Division of Gastroenterology and Hepatology, Houston Methodist Hospital, 6550 Fannin Street, SM 1001, Houston, Texas 770030, USA.
equigley@houstonmethodist.org

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Competing interests statement

The author declares no competing interests.