

# Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ

Caitriona M. Guinane and Paul D. Cotter

**Abstract:** The human gut microbiota has become the subject of extensive research in recent years and our knowledge of the resident species and their potential functional capacity is rapidly growing. Our gut harbours a complex community of over 100 trillion microbial cells which influence human physiology, metabolism, nutrition and immune function while disruption to the gut microbiota has been linked with gastrointestinal conditions such as inflammatory bowel disease and obesity. Here, we review the many significant recent studies that have centred on further enhancing our understanding of the complexity of intestinal communities as well as their genetic and metabolic potential. These have provided important information with respect to what constitutes a 'healthy gut microbiota' while furthering our understanding of the role of gut microbes in intestinal diseases. We also highlight recently developed genomic and other tools that are used to study the gut microbiome and, finally, we consider the manipulation of the gut microbiota as a potential therapeutic option to treat chronic gastrointestinal disease.

**Keywords:** gastrointestinal disease, gut health, microbial diversity, microbial manipulation

## Introduction

The human intestinal tract harbours a diverse and complex microbial community which plays a central role in human health. It has been estimated that our gut contains in the range of 1000 bacterial species and 100-fold more genes than are found in the human genome [Ley *et al.* 2006a; Qin *et al.* 2010]. This community is commonly referred to as our hidden metabolic 'organ' due to their immense impact on human wellbeing, including host metabolism, physiology, nutrition and immune function. It is now apparent that our gut microbiome coevolves with us [Ley *et al.* 2008] and that changes to this population can have major consequences, both beneficial and harmful, for human health. Indeed, it has been suggested that disruption of the gut microbiota (or dysbiosis) can be significant with respect to pathological intestinal conditions such as obesity [Ley *et al.* 2006b; Zhang *et al.* 2009] and malnutrition [Kau *et al.* 2011], systematic diseases such as diabetes [Qin *et al.* 2012] and chronic inflammatory diseases such as inflammatory bowel

disease (IBD), encompassing ulcerative colitis (UC) and Crohn's disease (CD) [Frank *et al.* 2007].

The role of the gut microbiome in human health and disease is becoming clearer thanks to high throughput sequencing technologies (HTS) as well as parallel recent developments in nongenomic techniques. The purpose of this review is to summarize the very significant major developments that have occurred with respect to revealing the microbial diversity of the human gut and how this intestinal microbiota impacts on gastrointestinal (GI) disease. We also discuss the state-of-the-art tools that can be used to study the gut microbiome and look to future therapeutic options, such as the manipulation of the gut microbiota, to address GI conditions.

## Tools for studying the gut microbiome

Understanding the composition and functional capacity of the gut microbiome represents a major

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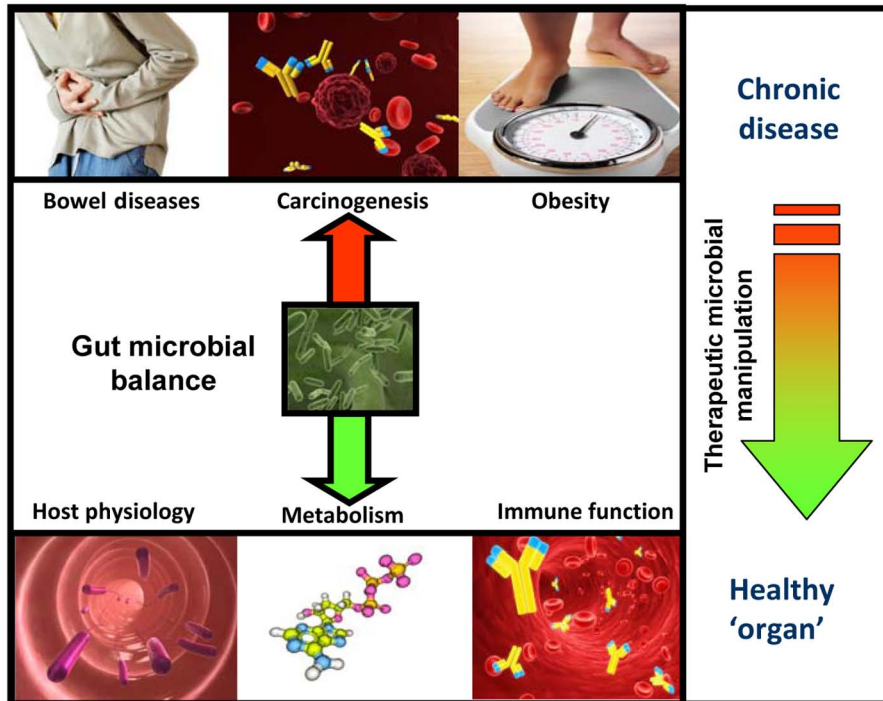
challenge. However, research in this area is ever expanding and currently a number of different approaches are being used and developed to determine gut microbial composition, genetic content and function.

Traditionally, culture-based techniques were used to determine the composition of the gut microbiota. These approaches have generally focused on the 'easy-to-culture' microbes of the gut and have become less popular due to indications that just 10–50% of the gut bacteria are culturable [Eckburg *et al.* 2005]. Culturing-based methods certainly have their limitations and do not readily provide an overview of the gut microbial composition. It should be noted, however, that there have been some advances in this area through the increased availability of specialized media to cultivate more fastidious organisms [Goodman *et al.* 2011]. A recent study constitutes a further development in this area and has resulted in the coining of the term 'microbial culturomics' [Lagier *et al.* 2012]. Microbial culturomics introduces an array of new culturing techniques, coupled with matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MS), to identify a range of previously uncultivated microbiota from the gut. This strategy includes the elimination of the 'easy-to-culture', or more abundant, populations that are present in high numbers to facilitate the enrichment of the more difficult to culture organisms by methods such as diverse filtration or the use of antibiotics and phage cocktails, leading to the identification of 174 species not previously described in the human gut [Lagier *et al.* 2012].

Despite these recent successes, it is clear that culture-independent approaches are better suited to providing a more rapid insight into the gut microbiota. In particular, the development and application of fast and low-cost DNA sequencing methods has been revolutionary. HTS has been widely used to examine the complexity of the gut microbiome due to the speed, scale and precise information provided. For compositional analysis, the 16S rRNA gene has been most frequently targeted due to its presence in all prokaryotes and the existence of variable domains that allow different taxa to be distinguished. Although the majority of HTS studies to date have relied on the Roche 454 pyrosequencing platforms, other sequencing technologies, such as those provided by Illumina (San Diego, CA, USA), are becoming more popular [Caporaso *et al.* 2011b]. Other HTS

technologies that can be applied include the SOLid system (Applied Biosystems, Foster City, CA, USA), the Ion platforms (Life Technologies, Carlsbad, CA, USA) and SMRT system (Pacific Biosystems, Menlo Park, CA, USA), while additional platforms, such as those that rely on nanopore technology, are in development [Clarke *et al.* 2009; Rosenstein *et al.* 2012; Schadt *et al.* 2010].

While 16S rRNA studies provide data in relation to the microbial composition of an ecosystem, these do not provide direct information regarding the microbial viability or the functional potential of the populations present. Metagenomic (or shotgun sequencing) studies go beyond the 16S rRNA gene to characterize the full genetic content of a community, thereby providing an insight into the potential functional capacity of the microbes present [Kurokawa *et al.* 2007; Qin *et al.* 2010; Turnbaugh *et al.* 2009b]. Regardless of the approach taken, it is important to note that these sequencing technologies require detailed bioinformatic analyses to deal with the large volumes of data generated (for a review, see Kuczynski and colleagues) [Kuczynski *et al.* 2012]. Indeed, increasingly, the major bottleneck has moved from being the generation of data to the storage of these data and the availability of scientists with the appropriate specialist bioinformatic skills. Furthermore, although these gene-centric approaches have provided much information regarding the content of the gut, we also need to understand the activity of these genes and the impact on the metabolic networks within the gut. To further determine specific microbial activity, it is necessary to analyse gene expression (metatranscriptomics), protein products (metaproteomics) and metabolic profiles (metabolomics). These techniques can be complex and, to different extents, are still somewhat in their infancy. To date, metatranscriptomics, based on large-scale sequencing of 16S rRNA transcripts, has been used to look at the composition of the active microbiota in healthy individuals and has revealed that the transcriptional profile across individuals is more similar than indicated by the associated taxonomic diversity [Gosalbes *et al.* 2011, 2012]. The faecal metaproteome of healthy adults was also recently investigated using liquid chromatography–tandem MS [Kolmeder *et al.* 2012]. Metaproteomics has an advantage over RNA-based studies as it analyses a more stable gene product. This study showed that the metaproteome retained



**Figure 1.** The gut microbiota in health and intestinal disease. The gastrointestinal microbiota play a role in host physiology, metabolism and nutrition. An alteration in the gut microbial community is linked to a number of intestinal conditions, including cancer, obesity and a variety of bowel disorders. The contribution of beneficial components of the gut microbiome to host physiology, metabolism and immune function has become the focus of ever more attention, and will undoubtedly lead to new therapeutic approaches.

considerable temporal stability over time and contained a proteome core that included metabolic enzymes, chaperones and stress proteins [Kolmeder *et al.* 2012]. The field of metabolomics has advanced dramatically and developments with respect to nuclear magnetic resonance (NMR) and MS make it possible to analyze 1000s of metabolites simultaneously [Nicholson *et al.* 2005]. NMR has been used to investigate metabolite compositions of the gut microbiota in very many instances [Marchesi *et al.* 2007; Mestdagh *et al.* 2012; Saric *et al.* 2008]. Although an extremely valuable tool, NMR can be limited by resolution and sensitivity. In some cases, ion cyclotron resonance–Fourier transform MS, which has an extremely high mass resolution and which can detect small variations between metabolite signals [Rossello-Mora *et al.* 2008], may merit consideration.

### Large-scale studies of the gut microbiome

In recent years a number of large funding initiatives were undertaken with a view to understanding the complexity of the human microbiome,

including the gut environment. The European Metagenomics of the human intestinal tract (MetaHIT) [Arumugam *et al.* 2011; Qin *et al.* 2010] and the US human Microbiome Project (HMP) [HMP Consortium, 2012a, 2012b] have both, through large-scale sequencing, worked towards establishing the baseline healthy gut microbiota and how this is altered in a disease state.

MetaHIT focused on investigating the correlation between the gut microbiome and intestinal pathologies, particularly obesity and IBD [Qin *et al.* 2010]. In one instance, this consortium sequenced faecal DNA from a cohort of 124 individuals, including healthy subjects and those with IBD or obesity, to establish a catalogue of nonredundant genes from the intestinal tract [Qin *et al.* 2010]. This project indicated that 40% of genes were shared among the majority of individuals and therefore represented a core metagenome. It was also found that 99.1% of genes were of bacterial origin, with the majority of the remaining genes belonging to the archaeal kingdom, with a relatively small number of eukaryotic and viral genes also being detected [Qin *et al.* 2010].

The HMP assessed the diversity of the microbiota across multiple body sites in healthy subjects, including the GI tract, to determine the baseline composition of the healthy human microbiome [HMP Consortium, 2012a]. Large-scale sequencing for meta-analyses has produced 16S rRNA data from 690 samples from 300 subjects and across 15 body sites [Turnbaugh *et al.* 2007]. The HMP also generated a catalogue of microbial genomes from the human microbiome, which consists of approximately 800 reference genomes from multiple body sites to date [HMP Consortium, 2012b] (see also <http://hmpdacc.org>). Both consortia provided a hugely valuable microbial catalogue that highlights the substantial variation in microbial species and genes in the gut. In addition, together with others, this work helps our understanding of what constitutes a 'healthy' gut microbiota while revealing novel potential associations between the gut microbiota and GI diseases [Qin *et al.* 2010, Arumugam *et al.* 2011; HMP Consortium, 2012a, 2012b].

### The 'healthy' gut microbiota

The intestinal microbiota of healthy individuals is known to confer a number of health benefits relating to, for example, pathogen protection, nutrition, host metabolism and immune modulation [O'Hara and Shanahan, 2006; Sekirov *et al.* 2010] (Figure 1). Historically, culture-based analysis has indicated that the gut of a healthy adult share bacterial species that are common among the majority of individuals. In contrast, however, the application of more recently developed technologies, which facilitate the culture-independent examination of the gut microbiota, have indicated that there is large interindividual microbial diversity, with only a small phylogenetic overlap between people [HMP Consortium, 2012a]. It should also be noted that the many HTS-based studies undertaken to describe the normal GI microbial community have differed with respect to the health, age, location and diet of the individuals included [Qin *et al.* 2010, 2012; Tap *et al.* 2009; Turnbaugh *et al.* 2009a], in the specific molecular methods used [Claesson *et al.* 2009; Hamady and Knight, 2009] and in how the data have been analysed [Wooley and Ye, 2009]. It has been established, however, that there is a high overall temporal stability of the microbial community within an individual, which suggests the existence of an individual core microbial population [Caporaso *et al.* 2011a; Costello *et al.* 2009; Jalanka-Tuovinen *et al.* 2011]. Even here, a

number of factors including aging, diet, antibiotic use and environmental factors can cause changes.

Infants are generally thought to be born with intestines that are sterile or that, at most, contain a very low level of microbes [Jimenez *et al.* 2008]. However, the infant GI tract is rapidly colonized following delivery. The composition of the infant gut can vary significantly based on a number of factors, including mode of delivery, feeding type, or due to antibiotic, prebiotic or probiotic use (for a review see Fouhy and colleagues) [Fouhy *et al.* 2012]. Despite this, the infant intestinal microbiota remains less complex than that of adults. Early colonizers include enterobacter and enterococci followed by anaerobic organisms such as bifidobacteria, clostridia, *Bacteroides* spp. and anaerobic streptococci [Adlerberth and Wold, 2009]. These populations continue to evolve and by age 2 the infant gut microbiota is thought to display a community structure similar to the adult gut [Palmer *et al.* 2007].

As noted above, interindividual variation within the adult gut microbiota is very large. Turnbaugh and colleagues established that the faecal microbiome of identical twins share less than 50% of species phylotypes [Turnbaugh *et al.* 2010]. However, based on HTS 16S rRNA-based, studies, it is apparent that in general the adult gut is dominated by two bacterial phyla, *Firmicutes* and *Bacteroidetes*, with other phyla including *Actinobacteria*, *Proteobacteria*, *Verrucomicrobia* and *Fusobacteria* being present in lower proportions [Eckburg *et al.* 2005; Tremaroli and Backhed, 2012]. Greater variations exist below the phylum level, although certain butyrate-producing bacteria, including *Faecalibacterium prausnitzii*, *Roseburia intestinalis* and *Bacteroides uniformis*, have been identified as key members of the adult gut microbiota [Qin *et al.* 2010]. Further knowledge relating to the species and functional composition of the gut was gleaned through the analysis of sequence data from 22 faecal metagenomes from individuals across four countries. This has led to a suggestion that the human gut microbiome consists of three enterotypes that vary with respect to the associated microbial species and their functional potential [Arumugam *et al.* 2011]. These clusters were named to reflect their dominant members, that is, *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3). It was claimed that the most frequent of these was enterotype 3, which is enriched in *Ruminococcus* in addition to the co-occurring

*Akkermansia* [Arumugam *et al.* 2011]. It has since been indicated, however, that the three enterotype divisions are not as distinct as first thought and in particular the *Ruminococcus*-dominant enterotype appears less evident than initially claimed [Jeffery *et al.* 2012a; Wu *et al.* 2011].

The elderly intestinal microbiota has also been the subject of a number of studies in recent years. This is particularly timely as an ageing population is now becoming a general feature of Western countries [Biagi *et al.* 2010; Claesson *et al.* 2011, 2012]. It has been noted that there are age-related physiological changes in the GI tract of older people that are characterized by a chronic low-grade inflammation (inflammageing) [Franceschi, 2007] which can lead to a microbial imbalance in the intestine [Guigoz *et al.* 2008]. HTS analysis has indicated that the composition of the gut microbiota of older people (>65 years) is distinct from that of younger adults and, although extremely variable between individuals, has a general dominance of the phylum *Bacteroidetes* [Claesson *et al.* 2011]. Claesson and colleagues further established a relationship between diet, the health status and the gut microbial population of older people [Claesson *et al.* 2012]. In summary, taxonomic assignments showed that the microbiota of people in a long-stay care environment had a high proportion of *Bacteroidetes*, whereas individuals living in the community had a high level of *Firmicutes*. Notably, the microbiota of individuals in long-stay care was significantly less diverse and a loss of the community-associated microbiota correlated with increased frailty [Claesson *et al.* 2012]. This and other work has strongly implied that the GI microbiota is extremely important to the health and in the progression of disease and frailty in older people [Claesson *et al.* 2012; Guigoz *et al.* 2008]. Regardless of age, the development of a clearer understanding of what constitutes a healthy microbiota allows one to establish what, if anything, is unusual within the microbiota of those with various diseases.

### The gut microbiota and disease

As the volume of data relating to the composition and functional potential of the gut microbiota increases, the number of diseases that have been linked with alterations in our gut microbial community has also expanded. Indeed, the many instances of such potential associations are too great to summarize in this review and thus here the focus is on associations that have been the

focus of greatest attention, that is, the possibility of a link between the gut microbiota and chronic GI diseases, including irritable bowel syndrome (IBS) and IBD, systemic diseases such as type 2 diabetes (T2D) and obesity, as well as the onset of colorectal cancer (CRC) (Table 1 and Figure 1).

#### *Irritable bowel syndrome*

Functional bowel disorders such as IBS are defined solely on symptom-based diagnostic criteria. IBS is characterized by abdominal pain or discomfort and altered bowel habits. Although the aetiology is multifactorial, recent understanding of the pathophysiology of IBS has revealed that variations in the normal gut microbiota may have a role to play in the low-grade intestinal inflammation associated with the syndrome [Brint *et al.* 2011; Ponnusamy *et al.* 2011]. Microbial dysbiosis in the gut is thought to be involved in IBS pathogenesis through facilitating adhesion of pathogens to the bowel wall (for a review, see Ghoshal and colleagues [Ghoshal *et al.* 2012]). Specifically, a study involving phylogenetic microarrays and quantitative polymerase chain reaction (qPCR) analysis revealed a clear separation between the GI microbiota of patients with IBS and that of the controls, that is, IBS was characterized by an increase in *Firmicutes* and, more specifically, in the numbers of *Ruminococcus*, *Clostridium* and *Dorea*, in addition to a marked reduction in *Bifidobacterium* and *Faecalibacterium* spp. [Rajilic-Stojanovic *et al.* 2011]. In a similar study of paediatric patients with the syndrome, an alteration in members of *Firmicutes* and *Proteobacteria*, also with a higher abundance of *Dorea*, *Ruminococcus* and *Haemophilus parainfluenzae*, was noted. Furthermore, members of the genus *Bacteroides* were found to be present at a lower level in paediatric patients with IBS than in the healthy controls and an increase in *Alistipes* was linked with a greater frequency of pain [Saulnier *et al.* 2011]. Other work by Jeffery and colleagues found subgroups among the patients with IBS with varying microbial signatures, however generally an increase in the *Firmicutes* to *Bacteroidetes* ratio was evident in patients with IBS who differed from normal populations [Jeffery *et al.* 2012b, 2012c]. These HTS studies suggest that a link between the gut microbiota and IBS may exist, which could, in time, lead to the design of therapeutic options.

#### *Inflammatory bowel disease*

IBD, encompassing both UC and CD, is characterized by a chronic and relapsing inflammation

**Table 1.** Microbial associations with chronic intestinal diseases.

Condition	Microbial association*	References <sup>§</sup>
IBS	Increased: <i>Firmicutes:Bacteroidetes</i> ratio <i>Ruminococcus</i> <i>Dorea</i> <i>Clostridium</i> <i>Gammaproteobacteria</i> (pIBS) <i>Haemophilus influenzae</i> (pIBS) Decreased: <i>Bifidobacterium</i> <i>Faecalibacterium</i> <i>Bacteroides</i>	Ghoshal <i>et al.</i> [2012]; Jeffery <i>et al.</i> [2012b]; Rajilic-Stojanovic <i>et al.</i> [2011]; Saulnier <i>et al.</i> [2011]
IBD (incl. CD and UC)	Increased: bacterial numbers in mucosa (CD) <i>Gamma-proteobacteria</i> <i>Enterobacteraceae</i> adherent invasive <i>Escherichia coli</i> (CD) <i>Clostridium</i> spp. Decreased: bacterial diversity <i>Firmicutes</i> <i>Bacteroidetes</i> <i>Lachnospiracheae</i> <i>Clostridium leptum</i> and <i>coccoides</i> group ( <i>Faecalibacterium prausnitzii</i> ) <i>Roseburia</i> <i>Phascolarctobacterium</i>	Frank <i>et al.</i> [2007]; Garrett <i>et al.</i> [2010]; Li <i>et al.</i> [2012]; Manichanh <i>et al.</i> [2006]; Morgan <i>et al.</i> [2012]
CRC	Increased: <i>Fusobacterium</i> spp. <i>E. coli</i> ( <i>pks+</i> )	Arthur <i>et al.</i> [2012]; Castellarin <i>et al.</i> [2012]; Kostic <i>et al.</i> [2012]; McCoy <i>et al.</i> [2013]
Obesity	Increased: <i>Firmicutes:Bacteroidetes</i> ratio <sup>‡</sup> <i>Actinobacteria</i> <i>Bacteroides</i> <sup>‡</sup> <i>Prevotellaceae</i> Decreased: bacterial diversity <i>C. leptum</i> group ( <i>Ruminococcus flavefaciens</i> ) <i>Bifidobacterium</i> <i>Methanobrevibacter</i>	Clarke <i>et al.</i> [2012]; Duncan <i>et al.</i> [2007]; Ley <i>et al.</i> [2005]; Schwieritz <i>et al.</i> [2009]; Turnbaugh <i>et al.</i> [2006, 2009a]; Zhang <i>et al.</i> [2009]
T2D	Increased: Opportunistic pathogens ( <i>Clostridium</i> spp., <i>E. coli</i> , <i>Eggerthella lenta</i> ) <i>Akkermansia muciniphilia</i> <i>Bacteroides</i> spp. Decreased: Butyrate-producing organisms ( <i>Roseburia</i> spp., <i>Faecalibacterium</i> spp., <i>Eubacterium</i> spp.) <i>Firmicutes</i>	Qin <i>et al.</i> [2012]; Larsen <i>et al.</i> [2010]
<p>*Examples of certain documented microbial changes associated with disease status.  <sup>§</sup>See also reviews by Cho and Blaser [2012]; Clarke <i>et al.</i> [2012]; Shanahan [2012].  <sup>‡</sup>Varying results among studies.  CD, Crohn's disease; CRC, colorectal cancer; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; pIBS, paediatric IBS; <i>pks+</i>, polyketide synthase positive; T2D, type 2 diabetes; UC, ulcerative colitis.</p>		

of the GI tract. UC and CD are generally described as chronic IBDs, although are distinct diseases that differ both in their symptoms and inflammation pattern. Specifically CD is a chronic, segmental inflammation of the GI tract [Loftus, 2004] and although the aetiology is not yet clear, it is defined as a complex trait that results from the interaction between the host genetics and the gut microbial population [Elson, 2002]. UC is generally characterized by inflammation and ulceration of the lining of the colon. The onset of both conditions is, in general, not thought to be due to a single causal organism but by a general microbial dysbiosis in the gut [Lepage *et al.* 2011; Martinez *et al.* 2008]. Nonetheless, this continues to be the subject of much debate. A role for gut microbes in the manifestation of IBD has been indicated by a number of studies and the gut microbiota are thought to be essential components in the development of mucosal lesions (for a review see Manichanh and colleagues) [Manichanh *et al.* 2012]. Intestinal inflammation is generally believed to be associated with a reduced bacterial diversity and, in particular, a lower abundance of, and a reduced complexity in, the *Bacteroidetes* and *Firmicutes* phyla with a specific reduction of abundance in the *Clostridium leptum* and *Clostridium coccoides* groups [Manichanh *et al.* 2006; Sokol *et al.* 2006]. It has also been indicated that while *Firmicutes* are reduced there is an increase in *gammaproteobacteria* in patients with CD [Li *et al.* 2012]. In contrast to the general microbial dysbiosis theory, some researchers have suggested the involvement of specific taxa, for example the *Enterobacteriaceae* have been associated with the microbiota of patients with UC [Garrett *et al.* 2010] and adherent invasive *E. coli* have been identified in the ileal mucosa of patients with CD [Darfeuille-Michaud *et al.* 2004]. There have been a number of studies that have also highlighted a lower abundance of *F. prausnitzii* (a member of the *C. leptum* group) in patients with CD and UC [Frank *et al.* 2007; Martinez-Medina *et al.* 2006; Sokol *et al.* 2009] and a role for this microorganism in combating bacterial dysbiosis in CD has been suggested [Sokol *et al.* 2008]. In addition, recent work analyzing intestinal biopsies and stool samples from patients with IBD and healthy subjects documented an association of the disease status of IBD with alterations in the abundances of *Enterobacteriaceae*, *Ruminococcaceae* and *Leuconostocaceae*, while at genus level, *Clostridium* levels increased whereas butyrate producer *Roseburia* and succinate producer *Phascolarctobacterium* were significantly reduced

in both UC and CD conditions [Morgan *et al.* 2012]. Regardless of the microbial population or pathogen in question, and although specific causality has not yet been clarified, these and other studies have certainly outlined a link between the gut microbiota and IBD.

#### Colorectal cancer

A role for the gut microbiome in the pathogenesis of CRC has been suggested in a number of recent publications [Arthur *et al.* 2012; Kostic *et al.* 2012; Plottel and Blaser, 2011]. Although a single causative organism has not been identified, a number of studies have implicated an association for *Fusobacterium* members with CRC [Castellarin *et al.* 2012; Kostic *et al.* 2012; McCoy *et al.* 2013]. More specifically, a recent study using fluorescent *in situ* hybridization analysis indicated a link between *Fusobacteria* and CRC, with higher numbers identified in tumours compared with control samples [Kostic *et al.* 2012]. This observation was supported by 16S rDNA sequencing analysis of the colorectal microbiome that revealed members of the *Fusobacterium* genus, including *Fusobacterium nucleatum*, *Fusobacterium mortiferum* and *Fusobacterium necrophorum* sequences, were enriched in tumour tissue. These changes were found to be accompanied by broad phylum-level changes, including a significant reduction in *Firmicutes* and *Bacteroidetes*. This may suggest that *Fusobacterium* spp. contribute to tumorigenesis through an inflammatory mechanism [Kostic *et al.* 2012]. Chronic inflammation is an established risk factor for carcinogenesis [Balkwill and Mantovani, 2001] and a tumour-associated or 'tumour-elicited' inflammation can be a feature of CRCs [Grivennikov *et al.* 2010]. Notably, another study, which relied on the use of metagenomic sequence and qPCR data, confirmed the association between this genus and CRC, revealing an overabundance of *Fusobacterium* sequences in tumour tissue compared with normal controls [Castellarin *et al.* 2012]. Members of *Fusobacterium*, interestingly, have also been associated with a number of other intestinal pathologies including IBD [Strauss *et al.* 2011] and acute appendicitis [Guinane *et al.* 2013; Swidsinski *et al.* 2011].

The link between microbially induced inflammation and CRC has also been highlighted in a number of other studies. Indeed it has been established that microbial products can enter barrier-defective colonic tumours, trigger inflammation through a host immune response and, in turn,

increase tumour growth [Grivennikov *et al.* 2012]. HTS studies have also revealed a link between inflammation and the gut microbial composition in colitis-susceptible, interleukin-10 deficient mice [Arthur *et al.* 2012]. This study revealed that mice with colitis had a less diverse gut microbial composition, which was accompanied by an increase in *Proteobacteria*, and particularly in *E. coli* levels, in the presence of intestinal inflammation [Arthur *et al.* 2012]. Ultimately, the role of some *E. coli* in CRC was linked to a polyketide synthase (*pks*) pathogenicity island encoding a genotoxin (colibactin). This was supported by the observations that isogenic mutants lacking the *pks* island brought about decreased tumour growth and invasion in mice than their wild-type *pks*<sup>+</sup> counterparts [Arthur *et al.* 2012]. Although these studies suggest that a combination of host inflammation and specific microorganisms contribute to CRC tumorigenesis, it is evident that further research in this area is needed.

#### *Obesity and type 2 diabetes*

Obesity and related disorders, such as T2D and metabolic syndrome, have become increasingly common in recent decades. Obesity is a complex syndrome that develops from a prolonged imbalance of energy intake and energy expenditure. Although lifestyle factors, diet and exercise contribute largely to the modern epidemic, it has also been indicated by an ever-increasing body of work that the microbial communities within the human intestine play an important role in obesity [Ley, 2010; Ley *et al.* 2005; Tilg and Kaser, 2011; Turnbaugh *et al.* 2006]. Although it has been suggested that increased energy harvest due to the presence of specific microbial populations contributes to obesity [Ley *et al.* 2005; Turnbaugh *et al.* 2006], this has not always been found to be the case [Murphy *et al.* 2010] and, indeed, it is becoming increasingly apparent that there can be very many other ways in which the microbiota can influence weight gain and host metabolism (for a review see Clarke and colleagues) [Clarke *et al.* 2012]. The identity of the key populations/taxa that may be associated with weight gain has also been the subject of much debate. Although a number of studies of the microbiota of lean and obese mice have indicated that genetically (*ob/ob*) and diet-induced obese mice contain higher proportions of *Firmicutes* and lower levels of *Bacteroidetes* than their lean counterparts [Ley *et al.* 2005], the situation in humans is less clear despite the fact that there have been a number of studies that have focused on the gut microbiota of lean and obese

individuals (for a review see Clarke and colleagues) [Clarke *et al.* 2012]. Indeed, Ley and colleagues, found a decrease in the *Firmicutes* to *Bacteroidetes* ratio following weight loss in human subjects [Ley *et al.* 2006b]. Further work by Turnbaugh and colleagues indicated a lower proportion of *Bacteroidetes* in obese individuals, an increased abundance of *Actinobacteria* while the levels of *Firmicutes* remained unaltered [Turnbaugh *et al.* 2009a]. The importance of the *Firmicutes* to *Bacteroidetes* ratio in obesity, however, is still not clear with some conflicting studies published to date in this area [Duncan *et al.* 2007; Schwartz *et al.* 2009].

T2D has, in recent years, become a health issue worldwide. T2D is principally linked with obesity-related insulin resistance. However, several genetic and environmental factors are thought to influence the condition. Here again, alterations in the composition of the gut microbiota of adults with T2D, relative to that of healthy controls, has been noted. Although in many instances the question as to whether these changes represent a cause or an effect remains unresolved, it is anticipated that further research in this area will clarify this issue. Regardless, a considerable number of fascinating studies have recently appeared. Larsen and colleagues employed 16S rRNA compositional sequencing to reveal that the proportions of the *Firmicutes*, and specifically the *Clostridia* class, were reduced, while the *Bacteroidetes* and the class *Betaproteobacteria* were enriched in a group with T2D compared with controls [Larsen *et al.* 2010]. More recently, an impressive large metagenome-wide association study identified gut microbial markers which might be useful in classifying T2D [Qin *et al.* 2012]. Overall, this study found a moderate degree of gut dysbiosis in patients with T2D. Of the identifiable bacterial species in this study it was indicated that control samples were enriched in various butyrate-producing bacteria, while patients with T2D were characterized by an increase in certain opportunistic pathogens, such as a number of *Clostridium* spp. in addition to important gut microbes including *Akkermansia muciniphilia*, *Bacteroides* spp. and *Desulfovibrio* spp. [Qin *et al.* 2012]. The identification of these gut microbial markers may be important in classifying T2D or perhaps other obesity or metabolic-related diseases.

#### **Strategies to manipulate the gut microbiota**

As shown in the above, there is growing evidence that the gut microbiota plays a central role in



human GI health and disease. It is therefore logical that modulating the gut microbiota should be considered as a therapeutic strategy to treat chronic disease. The approaches investigated include the use of prebiotics, supplementation with probiotics, reconstitution of bacterial populations by faecal transplantation or by employing antimicrobials to eliminate pathogens or manipulate the gut microbiota in a way that will benefit host health.

Prebiotics and probiotics are becoming increasingly popular (for a review see Vyas and Ranganathan) [Vyas and Ranganathan, 2012]. Prebiotics are nutritional compounds used to promote the growth of beneficial commensals and thus have the potential to improve GI health. Use of oral probiotic cultures to restore the gut microbiota has led to promising results in the treatment of intestinal disorders such as UC and obesity [Andreasen *et al.* 2010; Bibiloni *et al.* 2005; Kadooka *et al.* 2010]. While it can be argued, however, that oral probiotic doses do not provide sufficient microbial numbers to fully influence the populations of the colon, it may be that these microbes exert their influence through complex means, such as the production of an antimicrobial or a modulation of the immune system. Faecal microbial transplantation (FMT) is becoming a more commonly used approach to replenishing the GI microbiota (for reviews see Borody and Khoruts, and Floch) [Borody and Khoruts, 2011; Floch, 2012]. The aim of FMT is to reintroduce a stable community of GI microbes from a healthy donor to replace the disrupted populations in a diseased individual. In particular, FMT has been used in the treatment of recurrent *Clostridium difficile* infection when standard treatment has failed. FMT has been found to be successful in *C. difficile* treatment, with disease remission reported in up to 92% of cases [Gough *et al.* 2011].

In addition to being a viable therapeutic option, antibiotics can have potentially damaging effects through the perturbation of the gut microbiota. In particular, broad spectrum antibiotics can inflict significant ‘collateral damage’, as has been revealed recently by HTS technologies (for a review see Cotter and colleagues) [Cotter *et al.* 2012]. As a consequence, a number of investigations have focused on antimicrobials other than classical antibiotics. It is thus particularly notable that the ability to produce bacteriocins is a common feature among gut microbes. Bacteriocins are ribosomally synthesized small antimicrobial

peptides produced by bacteria with either a broad or narrow spectrum and to which the producing bacterium is immune [Cotter *et al.* 2005]. Bacteriocins with a narrow spectrum of activity against a target microorganism can offer a therapeutic alternative to traditional antibiotics. Gut-associated bacteriocin producers also have the advantage of producing the antimicrobial *in situ*, and therefore, in these situations, the antimicrobial peptide is not affected by proteolysis during gastric transit or does not need to be encapsulated. Bacteriocins have been shown to be useful in controlling a number of GI pathogens *in vivo*, including *Listeria monocytogenes* [Corr *et al.* 2007], *Salmonella* spp. [Casey *et al.* 2004], *Campylobacter jejuni* [Stern *et al.* 2006] and *C. difficile* [Rea *et al.* 2011].

In addition to employing antimicrobials with a view to controlling pathogens in the GI tract, it has also been suggested that antimicrobials could be employed to manipulate the microbiota to treat other GI disorders, such as obesity [Murphy *et al.* 2013a, 2013b]. In one instance, Murphy and colleagues explored the concept of targeting the gut microbiota diet-induced obese mice through two different antimicrobial strategies with a view to, in turn, assessing the impact on obesity-associated metabolic abnormalities [Murphy *et al.* 2013a]. The two interventions employed involved oral administration of the antibiotic vancomycin and the Abp 118 bacteriocin-producing probiotic *Lactobacillus salivarius* UCC 118 respectively. Both strategies altered the gut populations in distinct ways. For example, vancomycin administration resulted in a dramatic increase in *Proteobacteria* levels accompanied with a decrease in the *Firmicutes* and *Bacteroidetes* phyla, but only vancomycin resulted in an improvement in the metabolic abnormalities associated with obesity. These results further highlighted the link between the gut microbiota and health, and indicate the potential benefits of using gut microbiota-manipulating strategies to improve health [Murphy *et al.* 2013a, 2013b].

### Concluding remarks

Our gut microbiota evolves with us and plays a pivotal role in human health and disease. We now know that the resident microbiota influence host metabolism, physiology and immune system development while perturbation of the microbial community can result in chronic GI disease. While the revolution in molecular technologies

has provided us with the tools necessary to more accurately study the gut microbiota, we now need to more accurately elucidate the relationships between the gut microbiota and several intestinal pathologies. Understanding the part that microbial populations play in GI disease is fundamental to the ultimate development of appropriate therapeutic approaches. The concept of altering our gut community by microbial intervention in an effort to improve GI health is currently a topic that is receiving considerable interest. The targeting of specific components of the gut microbiome will potentially allow the removal of the harmful organisms and enrich the beneficial microbes that contribute to our health.

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### Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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