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

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24

25 **Abstract**

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

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49 **Introduction**

50 The human intestinal tract harbours a diverse and complex microbial community
51 which plays a central role in human health. It has been estimated that our gut
52 contains in the range of 1000 bacterial species and 100-fold more genes than are
53 found in the human genome [Ley *et al.*, 2006a, Qin *et al.*, 2010]. This community is
54 commonly referred to as our hidden metabolic 'organ' due to their immense impact
55 on human well-being, including host metabolism, physiology, nutrition and immune
56 function. It is now apparent that our gut microbiome coevolves with us [Ley *et al.*,
57 2008] and that changes to this population can have major consequences, both
58 beneficial and harmful, for human health. Indeed, it has been suggested that
59 disruption of the gut microbiota (or dysbiosis) can be significant with respect to
60 pathological intestinal conditions such as obesity [Ley *et al.*, 2006b, Zhang *et al.*,
61 2009] and malnutrition [Kau *et al.*, 2011], systematic diseases such as diabetes [Qin
62 *et al.*, 2012] and chronic inflammatory diseases such as Inflammatory Bowel Disease
63 (IBD), encompassing Ulcerative colitis (UC) and Crohns disease (CD) [Frank *et al.*,
64 2007].

65 The role of the gut microbiome in human health and disease is becoming
66 clearer thanks to high throughput sequencing technologies (HTS) as well as parallel
67 recent developments in non-genomic techniques. The purpose of this review is to
68 summarize the very significant major developments that have occurred with respect
69 to revealing the microbial diversity of the human gut and how this intestinal
70 microbiota impacts on gastrointestinal (GI) disease. We also discuss the state-of-the-
71 art tools that can be used to study the gut microbiome and look to future

72 therapeutic options, such as the manipulation of the gut microbiota, to address GI
73 conditions.

74 **Tools for studying the gut microbiome**

75 Understanding the composition and functional capacity of the gut microbiome
76 represents a major challenge. However, research in this area is ever expanding and
77 currently a number of different approaches are being used/developed to determine
78 gut microbial composition, genetic content and function.

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

96 Despite these recent successes, it is clear that culture-independent
97 approaches are better suited to providing a more rapid insight into the gut
98 microbiota. In particular, the development and application of fast and low cost DNA
99 sequencing methods has been revolutionary. HTS has been widely used to examine
100 the complexity of the gut microbiome due to the speed, scale and precise
101 information provided. For compositional analysis the 16S rRNA gene has been most
102 frequently targeted due to its presence in all prokaryotes and the existence of
103 variable domains that allow different taxa to be distinguished. Although the majority
104 of HTS studies to date have relied on the Roche 454 pyrosequencing platforms, other
105 sequencing technologies, such as those provided by Illumina are becoming more
106 popular [Caporaso *et al.*, 2011a]. Other HTS technologies that can be applied include
107 the SOLid system (Applied Biosystems), the Ion platforms (Life Technologies) and
108 SMRT system (Pacific Biosystems), while additional platforms, such as those that rely
109 on nanopore technology, are in development [Clarke *et al.*, 2009, Schadt *et al.*, 2010,
110 Rosenstein *et al.*, 2012].

111 While 16S rRNA studies provide data in relation to the microbial composition
112 of an ecosystem, these do not provide direct information regarding the microbial
113 viability or the functional potential of the populations present. Metagenomic (or
114 shotgun sequencing) studies go beyond the 16S rRNA gene to characterise the full
115 genetic content of a community, thereby providing an insight into the potential
116 functional capacity of the microbes present [Kurokawa *et al.*, 2007, Turnbaugh *et al.*,
117 2009a, Qin *et al.*, 2010]. Regardless of the approach taken, it is important to note
118 that these sequencing technologies require detailed bioinformatic analyses to deal
119 with the large volumes of data generated (for review see [Kuczynski *et al.*, 2012]).

120 Indeed, increasingly, the major bottleneck has moved from being the generation of
121 data to the storage of this data and the availability of scientists with the appropriate
122 specialist bioinformatic skills. Furthermore, although these gene-centric approaches
123 have provided much information regarding the content of the gut, we also need to
124 understand the activity of these genes and the impact on the metabolic networks
125 within the gut. To further determine specific microbial activity, it is necessary to
126 analyse gene expression (metatranscriptomics), protein products (metaproteomics)
127 and metabolic profiles (metabolomics). These techniques can be complex and, to
128 different extents, are still somewhat in their infancy. To date, metatranscriptomics,
129 based on large scale sequencing of 16S rRNA transcripts, has been used to look at
130 the composition of the active microbiota in healthy individuals and has revealed that
131 the transcriptional profile across individuals is more similar than indicated by the
132 associated taxonomic diversity [Gosalbes *et al.*, 2011, Gosalbes *et al.*, 2012]. The
133 faecal metaproteome of healthy adults was also recently investigated using liquid
134 chromatography-tandem MS [Kolmeder *et al.*, 2012]. Metaproteomics has an
135 advantage over RNA-based studies as it analyses a more stable gene product. This
136 study showed that the metaproteome retained considerable temporal stability over
137 time and contained a proteome core that included metabolic enzymes, chaperones
138 and stress proteins [Kolmeder *et al.*, 2012]. The field of metabolomics has advanced
139 dramatically and developments with respect to nuclear magnetic resonance (NMR)
140 and MS make it possible to analyse 1000s of metabolites simultaneously [Nicholson
141 *et al.*, 2005]. NMR has been used to investigate metabolite compositions of the gut
142 microbiota in very many instances [Marchesi *et al.*, 2007, Saric *et al.*, 2008,
143 Mestdagh *et al.*, 2012]. Although an extremely valuable tool, NMR can be limited by

144 resolution and sensitivity. In some cases, ion cyclotron resonance-fourier transform
145 MS (ICR-FT/MS), which has an extremely high mass resolution and which can detect
146 small variations between metabolite signals [Rossello-Mora *et al.*, 2008], may merit
147 consideration.

148 **Large scale studies of the gut microbiome**

149 In recent years a number of large funding initiatives were undertaken with a view to
150 understanding the complexity of the human microbiome including the gut
151 environment. The European Metagenomics of the human intestinal tract (MetaHIT)
152 [Qin *et al.*, 2010, Arumugam *et al.*, 2011] and the US human Microbiome Project
153 (HMP) [Human Microbiome Project Consortium 2012a; 2012b] have both, through
154 large scale sequencing, worked towards establishing the baseline healthy gut
155 microbiota and how this is altered in a disease state.

156 MetaHIT has focused on investigating the correlation between the gut
157 microbiome and intestinal pathologies, particularly obesity and IBD [Qin *et al.*, 2010].
158 In one instance, this consortium sequenced faecal DNA from a cohort of 124
159 individuals, including healthy subjects and those suffering from IBD or obesity, to
160 establish a catalogue of non-redundant genes from the intestinal tract [Qin *et al.*,
161 2010]. This project indicated that 40% of genes were shared among the majority of
162 individuals and therefore represented a core metagenome. It was also found that
163 99.1% of genes were of bacterial origin with the majority of the remaining genes
164 belonging to the archeal kingdom, with a relatively small number of eukaryotic and
165 viral genes also being detected [Qin *et al.*, 2010].

166 The HMP have assessed the diversity of the microbiota across multiple body
167 sites in healthy subjects, including the GI tract, to determine the baseline

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

180 **The 'healthy' gut microbiota**

181 The intestinal microbiota of healthy individuals is known to confer a number of
182 health benefits relating to, for example, pathogen protection, nutrition, host
183 metabolism, and immune modulation [O'hara and Shanahan, 2006, Sekirov *et al.*,
184 2010] (Figure 1). Historically, culture-based analysis has indicated that the gut of a
185 healthy adult share a 'core' microbiota with certain species being common to the
186 majority of individuals. In contrast, however, the application of more recently
187 developed technologies, which facilitate the culture-independent examination of the
188 gut microbiota, have indicated that there is large inter-individual microbial diversity,
189 with only a small phylogenetic overlap between people [Human Microbiome Project
190 Consortium, 2012a]. It should also be noted that the many HTS-based studies
191 undertaken to describe the normal GI microbial community have differed with

192 respect to the health, age, location and diet of the individuals included [Tap *et al.*,
193 2009, Turnbaugh *et al.*, 2009b, Qin *et al.*, 2010, Qin *et al.*, 2012], in the specific
194 molecular methods used [Claesson *et al.*, 2009, Hamady *et al.*, 2009] and in how the
195 data has been analysed [Wooley and Ye, 2009]. It has been established, however,
196 that there is a high overall temporal stability of the microbial community within an
197 individual which suggests the existence of an individual core microbial population
198 [Costello *et al.*, 2009, Jalanka-Tuovinen *et al.*, 2011, Caporaso *et al.*, 2011b]. Even
199 here a number of factors including aging, diet, antibiotic use and environmental
200 factors can cause changes.

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

212 As noted above, inter-individual variation within the adult gut microbiota is
213 very large. Turnbaugh and colleagues established that the faecal microbiome of
214 identical twins share less than 50% of species phylotypes [Turnbaugh *et al.*, 2010].
215 However, based on high throughput sequencing 16S rRNA-based, studies, it is

216 apparent that in general the adult gut is dominated by two bacterial phyla i.e.
217 *Firmicutes* and *Bacteroidetes*, with other phyla including *Actinobacteria*,
218 *Proteobacteria*, *Verrucomicrobia* and *Fusobacteria* being present in lower
219 proportions [Eckburg *et al.*, 2005, Tremaroli and Backhed, 2012]. Greater variations
220 exist below the phylum level, though certain butyrate-producing bacteria, including
221 *Faecalibacterium prausnitzii*, *Roseburia intestinalis* and *Bacteroides uniformis*, have
222 been identified as key members of the adult gut microbiota [Qin *et al.*, 2010].
223 Further knowledge relating to the species and functional composition of the gut was
224 gleaned through the analysis of sequence data from 22 faecal metagenomes from
225 individuals across 4 countries. This has led to a suggestion that the human gut
226 microbiome consists of 3 enterotypes that vary with respect to the associated
227 microbial species and their functional potential [Arumugam *et al.*, 2011]. These
228 clusters were named to reflect their dominant members i.e. *Bacteroides* (enterotype
229 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3). It was claimed that
230 the most frequent of these was enterotype 3, which is enriched in *Ruminococcus* in
231 addition to the co-occurring *Akkermansia* [Arumugam *et al.*, 2011]. It has since been
232 indicated, however, that the 3 enterotype divisions are not as distinct as first
233 thought and in particular the *Ruminococcus* dominant enterotype appears less
234 evident than initially claimed [Wu *et al.*, 2011, Jeffery *et al.*, 2012a].

235 The elderly intestinal microbiota has also been the subject of a number of
236 studies in recent years. This is particularly timely as an ageing population is now
237 becoming a general feature of Western countries [Biagi *et al.*, 2010, Claesson *et al.*,
238 2011, Claesson *et al.*, 2012]. It has been noted that there are age-related
239 physiological changes in the GI tract of the elderly that are characterized by a chronic

240 low-grade inflammation (inflammageing) [Franceschi, 2007] which can lead to a
241 microbial imbalance in the intestine [Guigoz *et al.*, 2008]. HTS analysis has indicated
242 that the composition of the gut microbiota of the elderly (>65 yrs) is distinct from
243 that of younger adults and, although extremely variable between individuals, has a
244 general dominance of the phylum *Bacteroidetes* [Claesson *et al.*, 2011]. Claesson *et*
245 *al.* further established a relationship between diet, the health status and the gut
246 microbial population of the elderly [Claesson *et al.*, 2012]. In summary, taxonomic
247 assignments showed that the microbiota of people in a long-stay care environment
248 had a high proportion of *Bacteroidetes*, whereas individuals living in the community
249 had a high level of *Firmicutes*. Notably, the microbiota of individuals in long-stay
250 care was significantly less diverse and a loss of the community-associated microbiota
251 correlated with increased frailty [Claesson *et al.*, 2012]. This and other work has
252 strongly implied that the GI microbiota is extremely important to the health and in
253 the progression of disease and frailty in the elderly [Guigoz *et al.*, 2008, Claesson *et*
254 *al.*, 2012]. Regardless of age, the development of a clearer understanding of what
255 constitutes a healthy microbiota allows one to establish what, if anything, is unusual
256 within the microbiota of those suffering from various diseases.

257 **The gut microbiota and disease**

258 As the volume of data relating to the composition and functional potential of the gut
259 microbiota increases, the number of diseases that have been linked with alterations
260 in our gut microbial community has also expanded. Indeed, the many instances of
261 such potential associations are too great to summarise in this review and thus here
262 the focus is on those associations that have been the focus of greatest attention i.e.
263 the possibility of a link between the gut microbiota and chronic GI diseases, including

264 Irritable bowel syndrome (IBS) and IBD, systemic diseases such as type 2 diabetes
265 (T2D) and obesity, as well as the onset of colorectal cancer (CRC) (Table 1) (Figure 1).

266 ***Irritable Bowel Syndrome***

267 Functional bowel disorders such as IBS are defined solely on symptom-based
268 diagnostic criteria. IBS is characterised by abdominal pain or discomfort and altered
269 bowel habits. Although the etiology is multifactorial, recent understanding of the
270 pathophysiology of IBS has revealed that variations in the normal gut microbiota may
271 have a role to play in the low-grade intestinal inflammation associated with the
272 syndrome [Brint *et al.*, 2011, Ponnusamy *et al.*, 2011]. Microbial dysbiosis in the gut
273 is thought to be involved in IBS pathogenesis through facilitating adhesion of
274 pathogens to the bowel wall (For review see [Ghoshal *et al.*, 2012]). Specifically, a
275 study involving phylogenetic microarrays and qPCR analysis revealed a clear
276 separation between the GI microbiota of IBS patients and that of the controls i.e. IBS
277 was characterised by an increase of *Firmicutes* and, more specifically, in the numbers
278 of *Ruminococcus*, *Clostridium* and *Dorea*, in addition to a marked reduction in
279 *Bifidobacterium* and *Faecalibacterium* sp. [Rajilic-Stojanovic *et al.*, 2011]. In a similar
280 study of paediatric patients with the syndrome, an alteration in members of
281 *Firmicutes* and *Proteobacteria*, also with a higher abundance of *Dorea*, *Ruminococcus*
282 and *Haemophilus parainfluenzae*, was noted. Furthermore, members of the genus
283 *Bacteroides* were found to be present at a lower level in paediatric IBS patients than
284 in the healthy controls and an increase in *Alistipes* was linked with a greater
285 frequency of pain [Saulnier *et al.*, 2011]. Other work by Jeffery *et al.*, found
286 subgroups among the IBS patients with varying microbial signatures, however
287 generally an increase in the *Firmicutes:Bacteroidetes* ratio was evident in IBS

288 patients who differed from normal populations [Jeffery *et al.*, 2012b, Jeffery *et al.*,
289 2012c]. These HTS studies suggest that a link between the gut microbiota and IBS
290 may exist, which could, in time, lead to the design of therapeutic options.

291 ***Inflammatory Bowel Disease***

292 IBD, encompassing both UC and CD, is characterised by a chronic and relapsing
293 inflammation of the GI tract. UC and CD are generally described as chronic IBDs,
294 although are distinct diseases that differ both in their symptoms and inflammation
295 pattern. Specifically CD is a chronic, segmental inflammation of the GI tract [Loftus,
296 2004] and although the etiology is not yet clear, it is defined as a complex trait that
297 results from the interaction between the host genetics and the gut microbial
298 population [Elson, 2002]. UC is generally characterised by inflammation and
299 ulceration of the lining of the colon. The onset of both conditions is, in general, not
300 thought to be due to a single causal organism but by a general microbial dysbiosis in
301 the gut [Martinez *et al.*, 2008, Lepage *et al.*, 2011]. Nonetheless, this continues to be
302 the subject of much debate. A role for gut microbes in the manifestation of IBD has
303 been indicated by a number of studies and the gut microbiota are thought to be
304 essential components in the development of mucosal lesions (for review see
305 [Manichanh *et al.*, 2012]). Intestinal inflammation is generally believed to be
306 associated with a reduced bacterial diversity and, in particular, a lower abundance of,
307 and a reduced complexity in, the *Bacteroidetes* and *Firmicutes* phyla with a specific
308 reduction of abundance in the *Clostridium leptum* and *Clostridium coccoides* groups
309 [Manichanh *et al.*, 2006, Sokol *et al.*, 2006]. It has also been indicated that while
310 *Firmicutes* are reduced there is an increase in *gamma-proteobacteria* in patients
311 with CD [Li *et al.*, 2012]. In contrast to the general microbial dysbiosis theory, some

312 researchers have suggested the involvement of specific taxa, for example the
313 *Enterobacteriaceae* have been associated with the microbiota of UC patients [Garrett
314 *et al.*, 2010] and adherent invasive *E. coli* have been identified in the ileal mucosa of
315 patients with CD [Darfeuille-Michaud *et al.*, 2004]. There have been a number of
316 studies that have also highlighted a lower abundance of *F. prausnitzii* (a member of
317 the *C. leptum* group) in patients with CD and UC [Martinez-Medina *et al.*, 2006, Frank
318 *et al.*, 2007, Sokol *et al.*, 2009] and a role for this microorganism in combating
319 bacterial dysbiosis in CD has been suggested [Sokol *et al.*, 2008]. In addition, recent
320 work analysing intestinal biopsies and stool samples from IBD and healthy subjects
321 documented an association of the disease status of IBD with alterations in the
322 abundances of *Enterobacteriaceae*, *Ruminococcaceae* and *Leuconostocaceae*, while
323 at genus level, *Clostridium* levels increased whereas butyrate-producer *Roseburia*
324 and succinate-producer *Phascolarctobacterium* were significantly reduced in both UC
325 and CD conditions [Morgan *et al.*, 2012]. Regardless of the microbial population or
326 pathogen in question, and although specific causality has not yet been clarified,
327 these and other studies have certainly outlined a link between the gut microbiota
328 and IBD.

329 ***Colorectal cancer***

330 A role for the gut microbiome in the pathogenesis of CRC has been suggested in a
331 number of recent publications [Plottel *et al.*, 2011, Arthur *et al.*, 2012, Kostic *et al.*,
332 2012]. Although a single causative organism has not been identified, a number of
333 studies have implicated an association for *Fusobacterium* members with CRC
334 [Castellarin *et al.*, 2012, Kostic *et al.*, 2012, McCoy *et al.*, 2013]. More specifically, a
335 recent study using Fluorescent In Situ Hybridization analysis indicated a link between

336 *Fusobacteria* and CRC, with higher numbers identified in tumours compared to
337 control samples [Kostic *et al.*, 2012]. This observation was supported by 16S rDNA
338 sequencing analysis of the colorectal microbiome that revealed members of the
339 *Fusobacterium* genus, including *Fusobacterium nucleatum*, *Fusobacterium*
340 *mortiferum*, and *Fusobacterium necrophorum* sequences, were enriched in tumour
341 tissue. These changes were found to be accompanied by broad phylum-level
342 changes, including a significant reduction in *Firmicutes* and *Bacteroidetes*. This may
343 suggest that *Fusobacterium* sp. contribute to tumourigenesis through an
344 inflammatory mechanism [Kostic *et al.*, 2012]. Chronic inflammation is an
345 established risk factor for carcinogenesis [Balkwill and Mantovani, 2001] and a
346 tumour-associated or 'tumour-elicited' inflammation can be a feature of colorectal
347 cancers [Grivennikov *et al.*, 2010]. Notably, another study, which relied on the use
348 of metagenomic sequence and qPCR data, confirmed the association between this
349 genus and CRC, revealing an overabundance of *Fusobacterium* sequences in tumour
350 tissue when compared to normal controls [Castellarin *et al.*, 2012]. Members of
351 *Fusobacterium*, interestingly, have also been associated with a number of other
352 intestinal pathologies including IBD [Strauss *et al.*, 2011] and acute appendicitis
353 [Swidsinski *et al.*, 2011, Guinane *et al.*, 2013].

354 The link between microbially-induced inflammation and CRC has also been
355 highlighted in a number of other studies. Indeed it has been established that
356 microbial products can enter barrier-defective colonic tumours, trigger inflammation
357 through a host immune response and, in turn, increase tumour growth [Grivennikov
358 *et al.*, 2012]. HTS studies have also revealed a link between inflammation and the
359 gut microbial composition in colitis-susceptible, interleukin-10 deficient, mice

360 [Arthur *et al.*, 2012]. This study revealed that mice with colitis had a less diverse gut
361 microbial composition, which was accompanied by an increase in *Proteobacteria*,
362 and particularly in *E. coli* levels, in the presence of intestinal inflammation [Arthur *et*
363 *al.*, 2012]. Ultimately, the role of some *E. coli* in CRC was linked to a polyketide
364 synthase (*pks*) pathogenicity island encoding a genotoxin (colibactin). This was
365 supported by the observations that isogenic mutants lacking the *pks* island brought
366 about decreased tumour growth and invasion in mice than their wild-type *pks*⁺
367 counterparts [Arthur *et al.*, 2012]. Although these studies suggest that a
368 combination of host inflammation and specific microorganisms contribute to CRC
369 tumourgenesis, it is evident that further research in this area is needed.

370 ***Obesity and Type-2 Diabetes***

371 Obesity and related disorders, such as T2D and metabolic syndrome, have become
372 increasingly common in recent decades. Obesity is a complex syndrome that
373 develops from a prolonged imbalance of energy intake and energy expenditure.
374 Although lifestyle factors, diet and exercise contribute largely to the modern
375 epidemic, it has also been indicated by an ever-increasing body of work that the
376 microbial communities within the human intestine play an important role in obesity
377 [Ley *et al.*, 2005, Turnbaugh *et al.*, 2006, Ley, 2010, Tilg and Kaser, 2011]. Although,
378 it has been suggested that increased energy harvest due to the presence of specific
379 microbial populations contributes to obesity [Ley *et al.*, 2005, Turnbaugh *et al.*, 2006],
380 this has not always been found to be the case [Murphy *et al.*, 2010] and, indeed, it is
381 becoming increasingly apparent that there can be very many other ways in which the
382 microbiota can influence weight gain and host metabolism (for review see [Clarke *et*
383 *al.*, 2012]). The identity of the key populations/taxa that may be associated with

384 weight gain has also been the subject of much debate. Although a number of studies
385 of the microbiota of lean and obese mice have indicated that genetically (*ob/ob*) and
386 diet-induced obese mice contain higher proportions of *Firmicutes* and/or a lower
387 levels of *Bacteroidetes* than their lean counterparts [Ley *et al.*, 2005], the situation in
388 humans is less clear despite the fact that there have been a number of studies that
389 have focussed on the gut microbiota of lean and obese individuals (for review see
390 [Clarke *et al.*, 2012]). Indeed, Ley and colleagues, found a decrease in the *Firmicutes*
391 to *Bacteroidetes* ratio following weight loss in human subjects [Ley *et al.*, 2006b].
392 Further work by Turnbaugh *et al.* indicated a lower proportion of *Bacteroidetes* in
393 obese individuals, an increased abundance of *Actinobacteria* while the levels of
394 *Firmicutes* remained unaltered [Turnbaugh *et al.*, 2009b]. The importance of the
395 *Firmicutes* and *Bacteroidetes* ratios in obesity, however, is still not clear with some
396 conflicting studies published to date in this area [Duncan *et al.*, 2007, Schwartz *et al.*,
397 2009].

398 Type-2 Diabetes has, in recent years, become a health issue worldwide. T2D
399 is principally linked with obesity-related insulin resistance. However, several genetic
400 and environmental factors are thought to influence the condition. Here again,
401 alterations in the composition of the gut microbiota of adults with T2D, relative to
402 that of healthy controls, has been noted. Although in many instances the question
403 as to whether these changes represent a cause or an effect remains unresolved, it is
404 anticipated that further research in this area will clarify this issue. Regardless, a
405 considerable number of fascinating studies have recently appeared. Larsen and
406 colleagues employed 16S rRNA compositional sequencing to reveal that the
407 proportions of the *Firmicutes*, and specifically the *Clostridia* class, were reduced,

408 while the *Bacteroidetes* and the class *Beta-proteobacteria* were enriched in a
409 diabetic, when compared to a control, group [Larsen *et al.*, 2010]. More recently, an
410 impressive large metagenome-wide association study (MGWAS) identified gut
411 microbial markers which might be useful in classifying T2D [Qin *et al.*, 2012]. Overall,
412 this study found a moderate degree of gut dysbiosis in patients with T2D. Of the
413 identifiable bacterial species in this study it was indicated that control samples were
414 enriched in various butyrate-producing bacteria, while patients with T2D were
415 characterised by an increase in certain opportunistic pathogens, such as a number of
416 *Clostridium* sp. in addition to important gut microbes including *Akkermansia*
417 *muciniphilia*, *Bacteroides* spp. and *Desulfovibrio* sp. [Qin *et al.*, 2012]. The
418 identification of these gut microbial markers may be important in classifying T2D or
419 perhaps other obesity or metabolic related diseases.

420 **Strategies to manipulate the gut microbiota**

421 As shown in the above, there is growing evidence that the gut microbiota plays a
422 central role in human GI health and disease. It is therefore logical that modulating
423 the gut microbiota should be considered as a therapeutic strategy to treat chronic
424 disease. Approaches investigated include the use of prebiotics, supplementation
425 with probiotics, reconstitution of bacterial populations by faecal transplantation or
426 by employing antimicrobials to eliminate pathogens or manipulate the gut
427 microbiota in a way that will benefit host health.

428 Prebiotics and probiotics are becoming increasingly popular (For review see
429 [Vyas and Ranganathan, 2012]). Prebiotics are nutritional compounds used to
430 promote the growth of beneficial commensals and thus have the potential to
431 improve GI health. Use of oral probiotic cultures to restore the gut microbiota has

432 led to promising results in the treatment of intestinal disorders such as ulcerative
433 colitis and obesity [Bibiloni *et al.*, 2005, Andreasen *et al.*, 2010, Kadooka *et al.*, 2010].
434 While it can be argued however that oral probiotic doses do not provide sufficient
435 microbial numbers to fully influence the populations of the colon, it may be that
436 these microbes exert their influence through complex means such as the production
437 of an antimicrobial or a modulation of the immune system. Fecal microbial
438 transplantation (FMT) is becoming a more commonly used approach to replenishing
439 the GI microbiota (For reviews see [Borody and Khoruts, 2011, Floch, 2012]). The
440 aim of FMT is to re-introduce a stable community of GI microbes from a healthy
441 donor to replace the disrupted populations in a diseased individual. In particular,
442 FMT has been used in the treatment of recurrent *Clostridium difficile* infection where
443 standard treatment has failed. FMT has been found to be successful in *C. difficile*
444 treatment with disease remission reported in up to 92% of cases [Gough *et al.*, 2011].

445 In addition to being a viable therapeutic option, antibiotics can have
446 potentially damaging effects through the perturbation of the gut microbiota. In
447 particular, broad spectrum antibiotics can inflict significant 'collateral damage', as
448 has been revealed recently in by HTS technologies (for review [Cotter *et al.*, 2012]).
449 As a consequence, a number of investigations have focused on antimicrobials other
450 than classical antibiotics. It is thus particularly notable that the ability to produce
451 bacteriocins is a common feature among gut microbes. Bacteriocins are ribosomally-
452 synthesised small antimicrobial peptides produced by bacteria with either a broad or
453 narrow spectrum and to which the producing bacterium is immune [Cotter *et al.*,
454 2005]. Bacteriocins with a narrow spectrum of activity against a target
455 microorganism can offer a therapeutic alternative to traditional antibiotics. Gut-

456 associated bacteriocin producers also have the advantage of producing the
457 antimicrobial *in situ* and therefore, in these situations, the antimicrobial peptide is
458 not affected by proteolysis during gastric transit or does not need to be
459 encapsulated. Bacteriocins have been shown to be useful in controlling a number of
460 GI pathogens *in vivo* including *Listeria monocytogenes* [Corr et al., 2007], *Salmonella*
461 sp. [Casey et al., 2004], *Campylobacter jejuni* [Stern et al., 2006] and *C. difficile* [Rea
462 et al., 2011].

463 In addition to employing antimicrobials with a view to controlling pathogens
464 in the GI tract, it is also now been suggested that antimicrobials could be employed
465 to manipulate the microbiota to treat other GI disorders, such as obesity [Murphy et
466 al., 2013a, Murphy et al., 2013b]. In one instance, Murphy et al. explored the
467 concept of targeting the gut microbiota diet-induced obese mice through two
468 different antimicrobial strategies with a view to, in turn, assessing the impact on
469 obesity-associated metabolic abnormalities [Murphy et al., 2013a]. The two
470 interventions employed involved oral administration of the antibiotic vancomycin
471 and the Abp 118 bacteriocin-producing probiotic *Lactobacillus salivarius* UCC 118,
472 respectively. Both strategies altered the gut populations in distinct ways, for
473 example vancomycin administration resulted in a dramatic increase in
474 *Proteobacteria* levels accompanied with a decrease in the *Firmicutes* and
475 *Bacteroidetes* phyla, but only vancomycin resulted in an improvement in the
476 metabolic abnormalities associated with obesity. These results further highlighted
477 the link between the gut microbiota and health and indicate the potential benefits of
478 using gut microbiota-manipulating strategies to improve health [Murphy et al.,
479 2013a, Murphy et al., 2013b].

480 **Concluding Remarks**

481 Our gut microbiota evolves with us and plays a pivotal role in human health and
482 disease. We now know that the resident microbiota influence host metabolism,
483 physiology and immune system development while perturbation of the microbial
484 community can result in chronic GI disease. While the revolution in molecular
485 technologies has provided us with the tools necessary to more accurately study the
486 gut microbiota, we now need to more accurately elucidate the relationships
487 between the gut microbiota and several intestinal pathologies. Understanding the
488 part that microbial populations play in GI disease is fundamental to the ultimate
489 development of appropriate therapeutic approaches. The concept of altering our
490 gut community by microbial intervention in an effort to improve GI health is
491 currently a topic that is receiving considerable interest. The targeting of specific
492 components of the gut microbiome will potentially allow the removal of the harmful
493 organisms and enrich the beneficial microbes that contribute to our health.

494

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499 **Figure Legend**

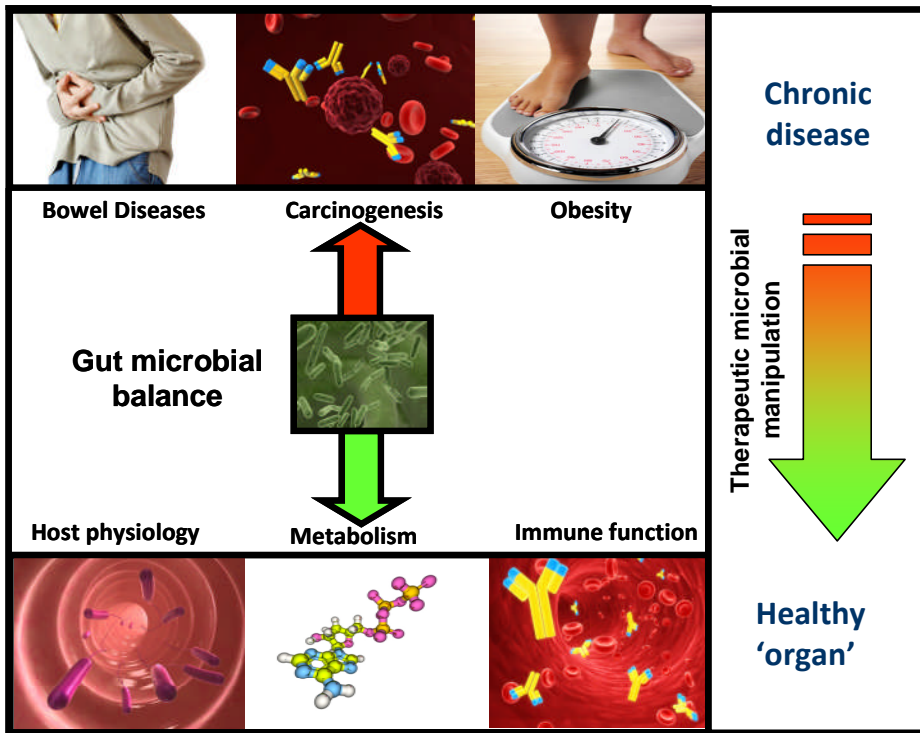
500 **Figure 1.** The gut microbiota in health and intestinal disease. The gastrointestinal
501 microbiota play a role in host physiology, metabolism and nutrition. An alteration in
502 the gut microbial community is linked to a number of intestinal conditions including
503 cancer, obesity and a variety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

507

508 **Figure 1**

509

510



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

Condition	*Microbial association	References#
IBS	<p>Increased: <i>Firmicutes:Bacteroidetes</i> ratio <i>Ruminococcus</i> <i>Dorea</i> <i>Clostridium</i> <i>Gamma-proteobacteria</i> (pIBS) <i>Haemophilus influenzae</i> (pIBS)</p> <p>Decreased: <i>Bifidobacterium</i> <i>Faecalibacterium</i> <i>Bacteroides</i></p>	[Rajilic-Stojanovic <i>et al.</i> , 2011, Saulnier <i>et al.</i> , 2011, Ghoshal <i>et al.</i> , 2012, Jeffery <i>et al.</i> , 2012b]
IBD (incl. CD and UC)	<p>Increased: bacterial numbers in mucosa (CD) <i>Gamma-proteobacteria</i> <i>Enterobacteraceae</i> adherent invasive <i>E. coli</i> (CD) <i>Clostridium</i> spp.</p> <p>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></p>	[Manichanh <i>et al.</i> , 2006, Frank <i>et al.</i> , 2007, Garrett <i>et al.</i> , 2010, Li <i>et al.</i> , 2012, Morgan <i>et al.</i> , 2012]
CRC	<p>Increased: <i>Fusobacterium</i> spp. <i>E. coli</i> (pks+)</p>	[Arthur <i>et al.</i> , 2012, Castellarin <i>et al.</i> , 2012, Kostic <i>et al.</i> , 2012, Mccoey <i>et al.</i> , 2013]
Obesity	<p>Increased: <i>Firmicutes:Bacteroidetes</i> ratio† <i>Actinobacteria</i> <i>Bacteroides</i> † <i>Prevotellaceae</i></p> <p>Decreased: bacterial diversity <i>C. leptum</i> group (<i>Ruminococcus flavefaciens</i>) <i>Bifidobacterium</i> <i>Methanobrevibacter</i></p>	[Ley <i>et al.</i> , 2005, Turnbaugh <i>et al.</i> , 2006, Duncan <i>et al.</i> , 2007, Schwartz <i>et al.</i> , 2009, Zhang <i>et al.</i> , 2009, Turnbaugh <i>et al.</i> , 2009b, Clarke <i>et al.</i> , 2012]

T2D	<p>Increased: Opportunistic pathogens (<i>Clostridium</i> spp., <i>E. coli</i>, <i>Eggerthella lenta</i>) <i>Akkermansia muciniphilia</i> <i>Bacteroides</i> spp.</p> <p>Decreased: Butyrate-producing organisms (<i>Roseburia</i> spp., <i>Faecalibacterium</i> spp., <i>Eubacterium</i> spp.) <i>Firmicutes</i></p>	<p>[Qin <i>et al.</i>, 2012] [Larsen <i>et al.</i>, 2010]</p>
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512 * Examples of certain documented microbial changes associated with disease status

513 † Varying results among studies

514 # See also reviews by [Cho and Blaser, 2012, Clarke *et al.*, 2012, Shanahan, 2012]

515 IBS- irritable bowel syndrome; IBD- Inflammatory Bowel Disease; CD- Crohn's Disease; UC-

516 Ulcerative colitis; CRC- colorectal cancer; T2D- Type-2 Diabetes; pIBS- paediatric IBS; pks+ -

517 polyketide synthase positive.

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