The gut microbiota and inflammatory bowel disease

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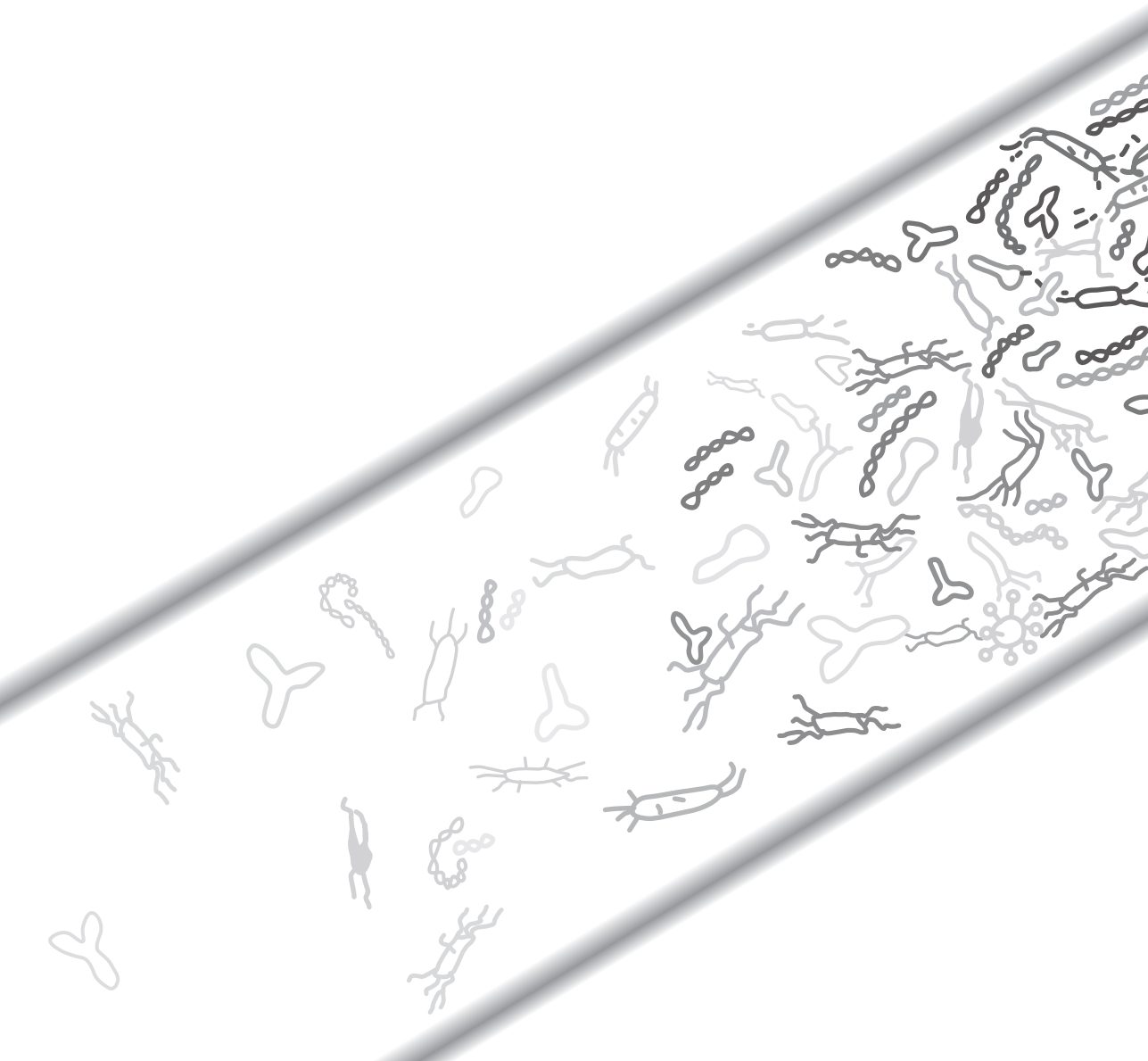
Discussion



In recent years, major steps have been made towards unravelling the pathogenesis of inflammatory bowel disease (IBD).[1] IBD has been associated to the host genetic architecture, the immune system and to environmental factors, all of which, in turn, interact with the gut microbiota.[2-11] Moreover, due to its potential for clinical application, the role of the gut microbiota in IBD is of great interest.[12] During my scientific journey as a PhD student, the microbiome field transitioned from low resolution associations to mechanistic studies. The availability of metagenomic sequencing has allowed us to better characterize the gut microbiota composition on a larger scale. This, in combination with extensive culturing of microbes using different culture conditions (i.e. culturomics), has given researchers the opportunity to identify different kinds of microbes.[13,14] As a result, the characterization of the gut microbiota in health and disease is becoming more and more detailed.

Within the context of the multifactorial disorder IBD, greater attention is being given to integrative models in which multiple facets of the pathogenesis of the disorder are studied simultaneously.[15-17] The Integrative Human Microbiome Project is a good example of a cohort with longitudinal data of multiple omics layers in patients with IBD.[18] Even though our knowledge about IBD and the role the gut microbiota plays in it has increased significantly, it is still very difficult to translate these findings towards clinical practice.

The goals of my thesis consisted of two parts. In part I (**Chapters 2** through **5**), the goal was to explore the role of the gut microbiota in the context of gastrointestinal disorders, more specifically in IBD and its subtypes. Considering my medical background, I am especially interested in investigating the clinical potential of these findings. Therefore, in part II (**Chapters 6** through **8**), I have incorporated the lessons learnt in part I into analysing whether the gut microbiota can be used in IBD management, specifically in the efficacy of medication use and the prediction of treatment outcomes. In **Chapter 9**, we set out our view on how to improve basic science in gut microbiota research and how to enable the clinical application of the gut microbiota in IBD. In this last chapter, I expand on this by discussing the main lessons learnt from the research within this thesis, as well as its limitations. I finish by discussing my perspectives on how the field needs to develop in order to translate knowledge about the gut microbiota towards clinical application in IBD.

Lessons learnt in this thesis

The gut microbiota in IBD – identifying an IBD signature

In **Chapter 2**, we show our efforts to identify a gut microbial signature in IBD (Figure 1). In concordance with previous research, this signature consists of a decrease in microbial richness,[19] a decrease in the relative abundance of anaerobic species and butyrate producers such as *Faecalibacterium prausnitzii* and *Bifidobacterium* species[20,21] and an enrichment of facultative anaerobes such as *Escherichia coli*[22,23] in comparison with the general population. Moreover, our results suggest that the increase in oxidative radicals resulting from inflammation gives facultative anaerobes an advantage. When analysing individual microbial pathways, we identified signatures indicative of a pro-inflammatory potential in IBD. This was, for example, indicated by a decrease of short chain fatty acid production.[24] Furthermore, various amino acids were decreased in IBD, including L-arginine. Supplementation of L-arginine improves inflammation in mice treated with dextran sulphate sodium to induce colitis.[25] Interestingly, both short chain fatty acids and L-arginine are depleted in the faecal metabolites of patients with IBD.[26] These findings indicate the importance of the identified pathways in the pathogenesis of IBD and provides one avenue for the identification of microbial-derived drug targets. Of note, a limitation here is that these findings are identified in cohorts containing patients who already had the diagnosis IBD, meaning that signals derived from these cohorts might reflect the impact of multiple disease-derived factors such as surgery of the intestine or exposure to IBD drugs. Validation of the results in, for example, treatment-naïve patients with IBD will provide more insight on this.

Considering the gut microbiota as an ecosystem

The gut microbiota forms an entire ecosystem, meaning that the microbes that collectively reside in our gut closely interact with each other and with the host. These microbes are also constantly exposed to environmental factors such as drugs.[27] A striking example of disruption of this ecosystem is shown by the overgrowth of the microbe *Clostridium difficile* after antibiotic therapy.[28] Currently, there is a lack of knowledge about how exactly these interactions occur and, therefore, about how the gut microbiota is collectively associated with health and disease.[29,30] Based on the findings of **Chapter 2**, **Chapter 3** moves beyond the discovery of independent altered microbial abundances in IBD to study how the microbes and microbial pathways interact with each other. By constructing co-abundance networks, we identified species involved in multiple IBD-specific co-abundance relationships. We hypothesized that these species are of importance in IBD pathogenesis. One example of such a species is *Escherichia coli*, which has been associated with IBD in previous studies. The abundance of *Escherichia coli* was positively correlated to *Streptococcus mutans* and negatively correlated to *Faecalibacterium prausnitzii*.[21,31] More importantly, these co-abundance relationships

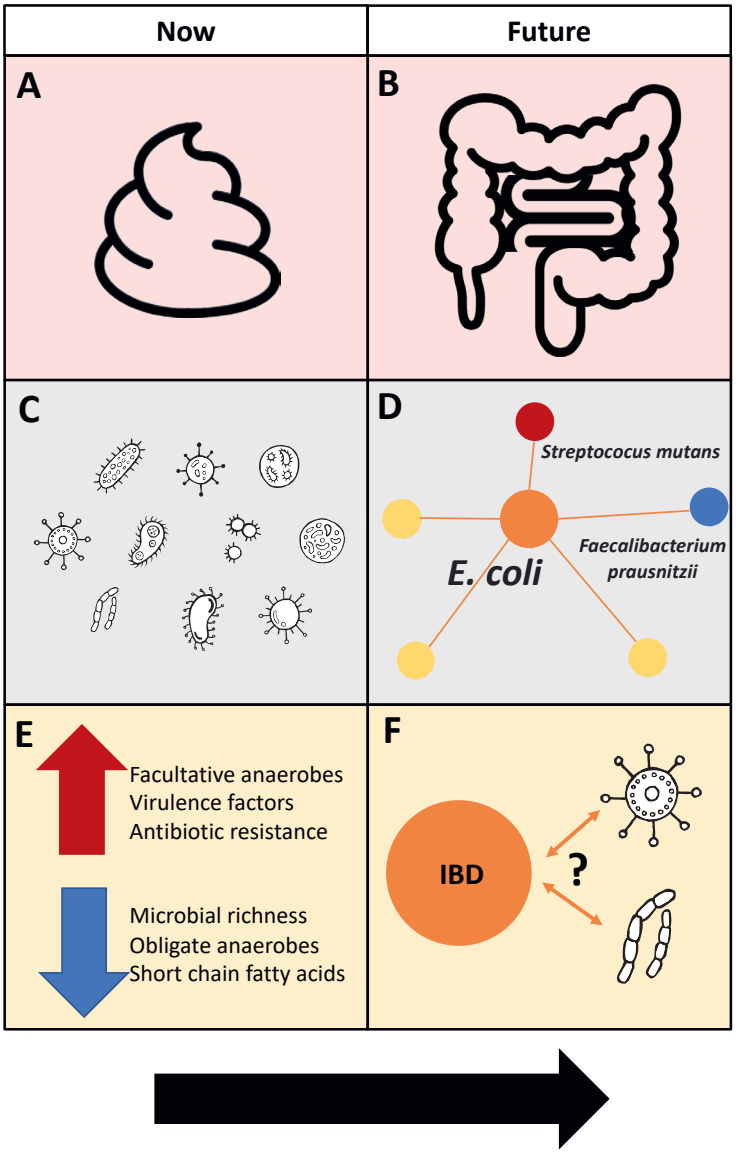


Figure 1. Current and future status of gut microbiota research in inflammatory bowel disease. Depicted are in panel A that currently by using faecal samples we mostly represent the colonic content of the gut microbiota, instead we should go to panel B representing the entire intestinal content. In panel C we should go from identifying individual microbes towards considering them as a gut ecosystem depicted in panel D. Finally, in E we should go from associations of the gut microbial findings towards panel F, i.e. causality.

were much weaker in non-IBD individuals, and adherent strains of *Escherichia coli* have been isolated in patients with IBD.[22] Therefore, we hypothesized that certain *Escherichia coli* strains induce immune responses, thereby creating a more pro-inflammatory environment that allows other opportunistic species to grow. *Escherichia coli* could potentially be of interest as a microbial target for IBD because treatments targeting this species might also restore the other microbes involved in the co-abundances. One reason for this is that the enrichment of these opportunistic species in faecal samples of patients with IBD is a consequence of the *Escherichia coli* immune reaction. However, the results of these studies must be interpreted with caution, since there is much ongoing debate about whether the construction of these co-abundance networks truly reflects the gut microbiota as an ecosystem.[32-34] While this debate is being resolved, the study of co-abundances can potentially prioritize taxa or pathways to be validated in follow-up functional studies.[35,36]

Studying the gut microbiota of the entire GI tract

One of the current limitations in understanding the gut microbiome is the use of faecal material. Although it is an accessible biomaterial that provides a lot of information about the host, it is not representative of the entire gastrointestinal tract. Previous research has shown that faecal material is highly enriched by colonic lumen bacterial content,[37] even though the small intestine is involved in numerous processes including food digestion and maturation of the immune system.[38,39] In **Chapter 4**, we studied metagenomes derived from faecal samples of IBD patients with an ileostomy or ileoanal pouch to examine the small intestinal microbiota. We identified that the diversities of the faecal gut microbiotas of patients with IBD were more similar to those of the small intestinal microbiota, as compared to that of the general population, and this was especially the case in patients with IBD who had undergone segmental resections. Furthermore, we identified that the species enriched in the small intestine are those usually found in the oral cavity, such as *Veillonella atypica* and *Streptococcus salivarius*.[40] We therefore hypothesized that the reduction in the microbial diversity in the colon in IBD could potentially lead to opportunistic growth of pathobionts from the small intestine, thereby playing a role in the pathogenesis of IBD. To disentangle which bacteria are specific to the small intestine or due to the IBD diagnosis, faecal samples from non-IBD patients with an ileostomy or intestinal biopsies are needed. In addition, studying intestinal biopsies from the same individuals, including from the upper part of the small intestine, would help to study the mucosa adherent microbiota of the small intestine in the future.

The need for replication

The lack of replication is a common issue in gut microbiota research and is a topic of debate within the field.[41-43] This low replication rate is especially the case in gene–microbiota interaction studies, indicating the need for replication studies in order to provide more

biological certainty about previously discovered findings.[44-46] In **Chapter 5**, our aim was to replicate the role of the *SLC39A8* missense variant in the gut microbiota composition.[47] We were able to replicate the finding that this missense variant was enriched in patients with CD compared to controls, but we were not able to replicate its association with the gut microbiota composition. This lack of replication could be due to the differences between the studies, for example the use of different type of samples. Considering that a large effect size of the gene-microbiota interaction was described in the discovery paper, we initially hypothesized that we should be able to replicate the findings despite these differences in study design. A striking example of this is the influence of the use of proton pump inhibitors on the gut microbiota composition, which – due to its large effect size – could be replicated in very different datasets containing humans and rats.[48-50] In my opinion, the focus of research should not only be based on "novelty", but also on replication and thereby validation of the findings, and scientific journals should therefore make room for a replication section.

Genetics, microbiota and drugs: a step forward to precision medicine

The development of new drugs is extremely expensive, reaching billions of euros for each new drug,[51] which makes increasing the efficiency of drug development a crucial goal. In **Chapter 6**, we analysed the use of genetic information for the identification of drug targets and, more importantly, for the identification of already existing drugs that might be repurposed for IBD management. We identified 113 drugs or compounds that have potential for use in IBD treatment, ranging from existing IBD drugs, to drugs used for other (inflammatory) indications, to compounds in testing phase. Following the example of the application of this methodology in rheumatoid arthritis,[52] our study shows how we can translate genetic findings towards clinical practice in IBD. Even though the effect size of a genetic risk variant is relatively small in IBD, the biological effect of targeting this variant by drugs can potentially be very large, as shown for HMG-CoA reductase inhibitors. These drugs have large effects on specific cholesterol levels in blood, while the genetic variants encoding HMG-CoA reductase have very small effects on the levels of this cholesterol.[53,54] In **Chapter 7**, we explored whether the gut microbiota has a predictive potential in IBD treatment. Our aim was to predict vedolizumab response based on the gut microbiome composition and levels of 92 circulating proteins.[55] We were able to predict treatment response with an area under the curve of 0.76 based on this information. However, we were not able replicate previous gut microbiota findings or our findings in an independent cohort.[56]

As described earlier, it is challenging to replicate microbiota studies, due to, amongst other factors, the lack of a golden standard in gut microbiota research.[41] In this case, another level of complexity was added by variability in the definition of treatment response. In the literature it is common to use disease severity scores, such as the Harvey

Bradshaw Index for CD and the Simple Clinical Colitis Activity Index for UC, to determine treatment response.[56-58] These scores are known to be subjective and do not fully capture whether the IBD is in an inflammatory state, thereby representing treatment response.[59-62] This could potentially mean that different cohorts have different definitions of response and non-response. I therefore propose we should consider the opinion of the treating physician, in this case meaning the determination of treatment response based on a combination of factors, including the disease severity scores, faecal calprotectin levels, laboratory values and clinical signs of showing response as determined by a treating physician who knows the patient best.

In **Chapter 8,** we explored the microbes-drug interaction from a different perspective. We aimed to study the influence of 41 commonly used drugs on the gut microbiota composition, since There is growing evidence that the gut microbiota also plays an important role in drug efficiency or the occurrence of side effects.[63,64] We identified that proton-pump inhibitors, laxatives and antibiotics showed the largest effects on the gut microbiota. This has also been shown *in vitro*, where these drugs inhibit the growth of specific bacterial species.[65] Furthermore, we identified an increase of the archaea *Methanobrevibacter smithii* in oral steroid users. This species is able to produce methane, which plays an important role in caloric harvest by facilitating the digestion of polyfructose.[66,67] This could potentially explain the frequently observed side effect of weight gain in the users of steroids and the association of this species with obesity and increased BMI in humans and rats.[66-68] Interestingly, different *Bacteroides* species have been shown *in vitro* to be involved in the metabolism of corticosteroids.[69] However, we could not find this association in our data, potentially due to the increase of *Bacteroides* species associated with IBD.

Future perspectives – improving upon current microbiota research

Setting standards and stimulating the scientific discussion within the microbiome field

Since the gut microbiota is highly variable and complex, the lack of a golden standard sampling method in microbiota research is introducing heterogeneity that is contributing to the reproducibility problem.[70] Faecal sample collection methods, such as freezing the sample immediately after production or the use of ethanol or RNALater as preserver, have an impact on the microbial composition. These methods lead to comparable alpha and beta diversity measurements; however, little is known about the effects of these different techniques on individual species.[71,72] Immediately freezing samples after production is considered good practise, since a low temperature slows bacterial growth,

thereby preserving the compositional state at the time of production as much as possible. Keeping the sample frozen, however, introduces logistical challenges, and this has led many studies to choose different ways to collect the faecal samples.[73]

During my PhD, I was also been involved in the logistical side of patient inclusion and the collection of faecal samples. We did opt for immediate freezing of the faecal sample at patients' homes and to keep the samples frozen until extraction of the microbial DNA, as depicted in Figure 2. Many steps are needed to go from faecal sample production to storage in the -80^{0} C freezer at the University Medical Center Groningen. In our research group, we have now used this method for over 14,000 faecal samples, and we still adhere it. In my opinion, considering the cost/effectivity in large-scale studies, this is the optimal method of faecal sample collection.

While faecal sample collection is only the first step in gut microbiota research, many more steps within this process still require standardization, including which DNA isolation kits to use, which computational steps need to be made to assign taxonomy and pathways, and which is the correct consequent statistical approach. Even though initiatives are ongoing to achieve more uniform practices,[18,74] we still have a long way to go. In my opinion, multiple changes have to be made to improve this. First, transparency is very important, including sharing of raw sequencing data and making the codes used publicly available. Furthermore, journals should make it more attractive for researchers to conduct replication studies, or, more importantly, for them to respond to already existing research to keep the scientific debate ongoing. Researchers should therefore get more time to respond to already existing research. The response to an article should not be perceived as a negative critique of other researchers' work, but rather as an open discussion and contraposition of ideas which is (or should be) in the scientific debate. *Gastroenterology*, a Q1 journal in the GI field, only allows researchers to respond on a published paper within a month after online publication. This timeframe limits the possibility for researchers to respond or conduct any replication study, thereby limiting scientific discussion.

The need for biobanks: multi-omics approaches in well-defined phenotypes

Biobanks with extensive phenotypes are important for research into the gut microbiota in IBD. Numerous environmental factors have been shown to influence gut microbiota composition in the general population, including diet, medication use and smoking.[5,9] Understanding this background is especially important in the context of IBD, where medications like proton pump inhibitors are regularly prescribed.[75] On top of that, as shown in **Chapter 2**, there are IBD-specific factors that also influence of the gut microbiota, such as intestinal resections and IBD-specific medications. Well-phenotyped biobanks are therefore needed to take these intrinsic and extrinsic factors into account and, more importantly, to provide the ability to assign gut microbial changes to different

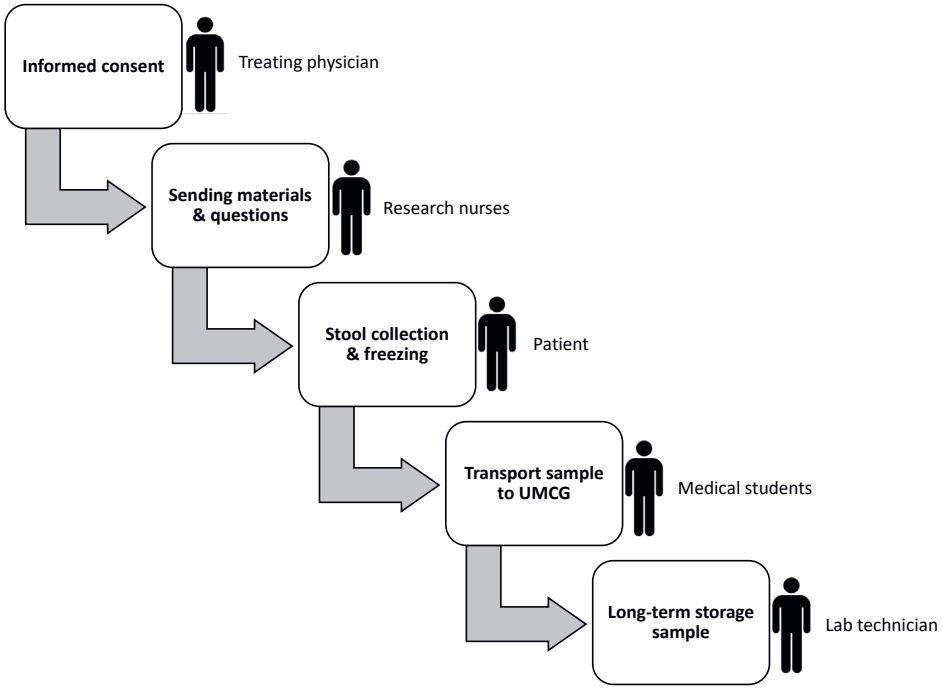


Figure 2. Process of stool sample collection and involved individuals.

IBD subtypes. In **Chapter 2**, for example, we observed that the gut microbial changes are more pronounced based on the disease location rather than the separate CD and UC diagnoses. The same has been shown in findings with regards to the genetic architecture of IBD.[7,76] In my opinion, we should take both the disease location and the separate diagnosis CD and UC into account in IBD research. This would potentially lead to microbiota-directed treatment strategies based on disease location.

Furthermore, it is becoming clearer that the gut microbiota engages in complex interactions with the host. Therefore, we need an integrative approach in which multiple data layers are collected for each individual patient, the so-called multi-omics approach. This will capture the multiple players that are involved in the pathogenesis of IBD, i.e. host genetics, the immune response, the gut microbiota and environmental factors. The Human Microbiome Project is a good example of a project in which multiple omics levels, including metagenome, metatranscriptome, proteome, metabolome and virome have been collected within the same IBD patients at multiple timepoints.[18]

During my PhD, I have been involved in the development of the 1000IBD cohort, which aims to collect multi-omics data for 1000 patients with IBD. Data collection for this

project is still ongoing.[77] Even though the creation of these cohorts is very useful, being attached to a university hospital means that we miss out on samples from some very interesting groups of patients with IBD and controls. In IBD, we usually miss treatment-naïve patients or patients with a relatively mild disease course, who are unlikely to be referred to a tertiary center for care. For the control group, it would be beneficial to also capture biomaterials such as intestinal biopsies from individuals with a positive occult blood test, or from individuals with gastrointestinal complaints, to rule out the influence of IBD. In my opinion, a wider range of medical doctors (including general practitioners and gastroenterologists from non-university hospitals) should be more involved in patients' enrolment and sample collection. Furthermore, we should also collect more biomaterials from all participants entering phase I/II/III trials for which clinical approval for certain drugs has been given. In the case of vedolizumab, for example, it would have been very useful to compare the data from our patients from **Chapter 7** to that of healthy volunteers taking vedolizumab in order to disentangle the effect on inflammation from the effect of the drug.

The role of the gut microbiota in IBD: cause or consequence?

Large cross-sectional studies have been very useful for gaining insights into the role of the gut microbiota in IBD. However, they provide very limited power to infer causality. Are the gut microbial changes observed in patients with IBD the cause or a consequence of the disease? The observed increase in aerotolerant species in IBD implies that gut microbial alterations are a consequence of the disease, given that inflammation leads to an increase of oxidative stress.[22] The transfer of human gut microbiota from patients with IBD, however, has been shown to induce an exacerbation of colitis in germ-free mice, which indicates that the gut microbiota could also be the cause of IBD.[78] The first longitudinal studies have helped to shed more light into the dynamic nature of the gut microbiota. One example is that the gut microbiota of patients with IBD shows more gut microbial compositional fluctuations than healthy individuals.[18,79] Within our research group, we have started a longitudinal study called IBD tracker that is following 50 patients with IBD and frequently collecting faecal samples, with the goal of shedding more light on gut microbial changes, especially in active disease.

In my opinion, we should include a variety of individuals in these longitudinal studies: 1) established patients who have had IBD for a longer time period in order to capture differences in disease activity and other heterogenous features of IBD, 2) patients with new onset IBD to gain more knowledge on the influence of IBD management such as different drug use and surgery, 3) birth cohorts such as Lifelines NEXT[80] to capture microbial perturbations early on in life, and 4) large-scale general population cohorts to capture pre-IBD and post-IBD disease states and to gain insight into the fluctuations of the gut microbiota composition associated with the general population. Finally, within our

research group, we also study IBD twins and their gut microbiota in order to investigate genetic, environmental and gut microbial factors.

Despite these efforts in functional and longitudinal studies, no evident cause-effect relationship has yet been established between the gut microbiota and IBD, for several reasons. One of the main limitations of functional approaches is that experiments are either performed in bacterial cultures or in animal models that do not fully reflect the human body. Since both the gut microbiota and the diagnosis IBD show large differences between individuals, using mice with uniform genetic make-ups and gut microbiota compositions does not reflect this complexity. New gut-on-a-chip technologies are promising methods to validate the findings. With this technology, human cells are used to create gut tissue, and it will be possible to introduce multiple microbes in this system at a later stage.[81] The ideal experimental set-up here would be to test IBD and its interaction with multiple facets of IBD aetiology, e.g. to create gut tissue with an IBD genetic background then add the gut microbiota and other environmental factors such as drugs. Furthermore, the longitudinal studies in IBD thus far have mostly been performed in IBD subgroups, for example the majority of patients with IBD from the HMP cohort were paediatric IBD patients.[18]

To truly study the entire gut microbiota, move beyond the "bacteriome"

Even though metagenomic sequencing has the potential to sequence the entire genetic content of the gut microbiome, thereby identifying bacteria, viruses, archaea and fungi, very little is known about the other members of the gut microbiota besides bacteria.[82] When using the terms "virome AND IBD" on Pubmed (search June 25th 2020), only 20 hits appeared, compared to 2,113 hits when entering the terms "microbiome AND IBD". Yet it is estimated that there are 10 times more bacteriophages than bacteria in the human gut microbiome.[83] Moreover, viruses have been linked to IBD pathogenesis, for example the alteration of bacterial functions by viruses, as is shown in *Faecalibacterium prausnitzii*, a bacterium that is decreased in IBD. Phages entering *Faecalibacterium prausnitzii* could even worsen the observed depletion of this bacterium in patients with IBD.[84] Additionally, dysbiosis based on the fungal microbiota has been identified in patients with IBD.[85] Therefore, when studying the gut microbiota in IBD, more emphasis and focus should be placed on the currently under-studied members of the gut microbiota.[86]

Future perspectives – towards clinical application

Hypes & hopes of the gut microbiota

The gut microbiota has received a lot of recent attention and has been linked to numerous diseases, ranging from disorders within the GI tract to psychiatric disorders.[87,88] Private

companies are now offering to analyse stool samples and make recommendations with regards to diet and protection against diseases based on the results.[89] However, there is scepticism about this, and critics have highlighted that we have not yet identified the underlying causal relationships, we lack a definition of a healthy microbiota and we are missing information on how exactly factors like diet alter the gut microbiota composition.[41] It is thus important to be aware of the gaps in current gut microbiota knowledge before it can be used in clinical practice. While we are still in an early stage of the implementation of the gut microbiota as a therapeutic option, it has a great potential for other clinical application as depicted in Figure 3, and in the following section I will elaborate on this.

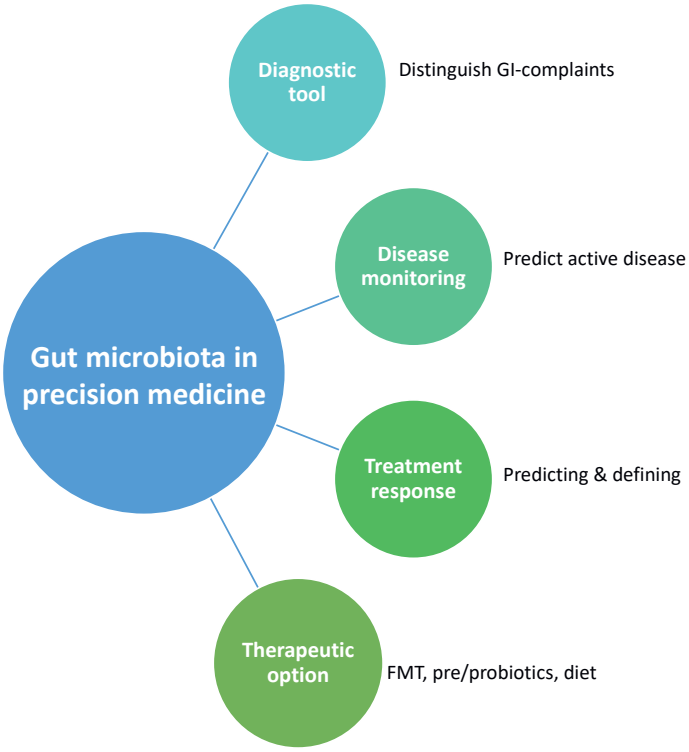


Figure 3. Clinical applications of the gut microbiota in inflammatory bowel disease.

The gut microbiota as a diagnostic tool or a tool to monitor disease course

In **Chapter 2**, we were able to use the gut microbiota to distinguish patients with IBD from members of the general population and, even more clinically relevant, from patients with irritable bowel syndrome (IBS). Others have also shown the predictive potential of the gut microbiota in IBD by using 16S rRNA sequencing or metabolomics data.[26,35]

Currently, in clinical practice, an endoscopy is needed to set the diagnosis IBD or IBS.[90] The use of gut microbiota information derived from stool samples thus has potential as a non-invasive diagnostic tool,[91,92] especially in excluding the IBD diagnosis in patients with IBS. A limitation of these studies, however, is that these findings are either identified in already established IBD cohorts or are predicting the IBD diagnosis from individuals without gastrointestinal complaints. Therefore, further validation is needed that better mimics the clinical context before applying this tool in clinical practice. Collaborations with general practitioners and non-university hospitals throughout the Netherlands would create a great opportunity to properly test this tool in the right individuals, meaning patients with new onset IBD and individuals with gastrointestinal complaints.

Once the diagnosis of IBD is made, the gut microbiota could be used as tool for monitoring disease course. Within our research group, we have shown that certain microbial pathways were decreased during exacerbations in patients with CD, e.g. there is a decrease in the biosynthesis of the vitamins riboflavin and thiamine, which are both known to have anti-inflammatory properties.[93] Furthermore, it has been shown longitudinally that active disease is linked to gut microbial perturbations.[18,79] The gut microbiota can therefore be used to monitor IBD patients in the same way that we currently use faecal calprotectin. At individual level, the most applicable way to do so would be to analyse fluctuations within a patient instead of detecting specific species or pathways.

Another interesting approach that needs further validation is the use of untargeted metabolomic platforms to identify molecules that can help in detecting microbial dysbiosis or disease activity. As I discussed in the introduction of this thesis, the gut microbiota is involved (together with the host) in the human metabolism. Residuals of metabolism, either small molecules or metabolites, can be measured in the faecal samples. In a small subset of IBD patients of the 1000IBD cohort and a cohort in the United States, researchers found that IBD-associated dysbiosis also has consequences that can be seen in the faecal metabolites. Considering that some of these metabolites are more easily quantified than the gut microbiota, this opens up the opportunity to use metabolites for cost-effective diagnosis and monitoring of IBD.

Predicting and/or defining treatment response

A striking example of how the gut microbiota can be used to predict treatment response is the case of immune checkpoint inhibitors. This relatively new therapy is used in a variety of cancers and, interestingly, its working mechanism shows similarities with the pathogenesis of IBD by stimulating the immune response mediated by T-lymphocytes. The goal of immune checkpoint inhibitor therapy is that this immune response targets the tumour cells, but a common side effect of this therapy is colitis.[94,95] The bacteria *Bacteroides* and *Bifidobacterium* were linked to an improved treatment response in mice

receiving checkpoint inhibitor treatment.[96,97] Identifying differences in the gut microbiota composition before start of a therapy has proven to be more difficult in IBD. Even though an enrichment of *Roseburia inulinivorans* and *Burkholderiales* species has been identified before the start of vedolizumab treatment in patients with CD who responded to the therapy,[63] we were not able to replicate this in **Chapter 7**. In my opinion, using the gut microbiota to predict response to biologicals is not suited to IBD. The inflammatory state patients with IBD are in along with the accompanying gut microbial changes makes it challenging to distinguish responders from non-responders. However, the gut microbiota is better suited for defining treatment response, since we did identify that the alpha diversity increased after 14 weeks in the IBD patients responding to vedolizumab treatment and this increase has also been observed in patients with IBD using other biologicals.[98] In this way, the gut microbiota can function as an additional way to determine treatment response in a non-invasive matter.

Using the gut microbiota as a therapeutic option

Faecal microbiota transplantation

Based on the conception of the microbiota as an organ, some researchers have asked if it was possible to transplant a "healthy microbiota" into someone with an unhealthy microbiota. This procedure, known as faecal microbiota transplantation (FMT), consists of the administration of stool from a "healthy" donor to a patient suffering from a gastrointestinal disorder associated to the gut microbiota, either through a nasoduodenal tube or by infusion in the colon.[99] While there is no evidence of its wide use, FMT has proven to be a successful treatment for *Clostridium difficile* infections that restores the microbial diversity in the gut.[100] In IBD, FMT has shown more beneficial results in UC than in CD.[101,102] In a large randomized controlled trial, 27% of patients with active UC reached remission after FMT, as compared to 8% in the placebo group.[101] Despite these results, there is currently not enough support for FMT in IBD management according to the current guidelines. This is mostly due to uncertainties regarding the selection of the right donors and about the frequency and (long-term) safety of the procedure.[103] The clinical trials performed so far have shown that FMT is thought to be a safe procedure; however, there are case reports in IBD where severe side effects of FMT, such as bacteraemia, have been described.[104] Before implementing FMT in IBD treatment, more research needs to be performed, e.g. clinical trials that compare FMT with a golden treatment standard, such as mesalamine use in UC. Further studies should also include combining IBD drugs with FMT to see whether FMT has an additive effect on standard IBD treatment.

Prebiotics & probiotics

Administering live microbes to benefit the health of the host, in other words the use of probiotics, has been studied for years. It has been shown that these probiotics are

involved in the immune function, produce antimicrobial compounds, show interactions with commensals and improve the barrier function of the gut.[105] In addition, the substrate utilized by these probiotics, so-called prebiotics, have also been suggested for use in the treatment of numerous diseases.[106] Patients with IBD have shown interest in these products, as illustrated by the fact that up to 74% of the patients with IBD have used prebiotics and probiotics at least once in a five-year period.[107] Many prebiotics and probiotics have shown effectiveness in animal models representing IBD, for example the anti-inflammatory properties of *Lactobacillus acidophilus* and L-arginine have been shown in IBD mice.[108,109] However, studies in humans have shown disappointing results of these products in IBD. In CD, multiple randomized controlled trials have been performed, and none have induced or maintained remission.[110,111] In pouchitis patients with UC, the probiotic mixture VSL#3 did show beneficial effects when combined with mesalamines.[112] Many more studies in larger sample sizes are therefore needed to test the same species, dosages and long-term effects before conclusions can be made about the potential role of prebiotics and probiotics in the treatment of IBD.

Diet

Patients commonly raise the question of which diet best suits their symptoms, but this remains a difficult question for clinicians to answer. More evidence is becoming available linking dietary components and inflammation. One example is dietary fibre. Beneficial short chain fatty acids such as butyrate are derived from dietary fibre, and high fibre has been linked to a diverse microbiota.[113] This is especially the case in the context of IBD, in which butyrate producers are depleted, and clinical trials with dietary fibres have been suggested.[114] Since the diet is a sum of individual nutrients, a few clinical trials have been conducted in patients with IBD studying whole diets. For example, a FODMAP diet lasting for 4 weeks showed beneficial effects in patients with quiescent IBD for their persistent gut symptoms.[115] The Mediterranean diet also seems to play an important role in preventing IBD, as participants adhering to this diet showed a lower risk of developing later onset CD.[116] Studying diet comes with its own challenges since it is not possible to include a placebo control and there are difficulties in acquiring dietary information and in following the complex interactions between foods and different food metabolisms in each individual. Dietary studies in large cohorts of patients with IBD are needed to study which dietary components work best for which IBD subtypes. Supplying food boxes containing dietary components with anti-inflammatory properties is a work in progress in our research department that aims to get a closer look of the role of diet as therapeutic option in IBD.

Towards precision medicine in IBD using the gut microbiota

The gut microbiota has great potential for use in precision medicine in IBD. Setting the right treatment for each individual should not only consist of gut microbial targets,

as shown by the lack of efficiency in reaching IBD remission based on FMT, probiotics, prebiotics and diet. It should be an important addition to the current immunosuppressive strategies. Considering the heterogeneous nature of IBD, a one-size-fits-all approach is not suited for this indication. Large studies are needed in which stratification of patients with IBD can be made based on, for example, disease location or the occurrence of intestinal resections in order to assess which microbial targets in combination with which immunosuppressive agents works best. Given that IBD is a multifactorial disease – meaning that genetics, the immune response and other environmental factors all play a role in its pathogenesis – stratification based on these factors should be useful in designing the best therapeutic strategies. In addition to guiding treatment of IBD, I also see a role of the gut microbiota in other parts of IBD precision medicine, including prevention, diagnosis and monitoring of disease activity. In our research group, we are currently analysing the faecal metagenomes of >8000 individuals from the general population with the aim of defining a "healthy" microbiota as part of the Dutch Microbiome Project. This will help shed light on the prevention of IBD based on the gut microbiota. The use of the gut microbiota in clinical practice, including in IBD, is still in its infancy, and, as described in this Discussion, much research needs to be done in order to properly apply the gut microbiota in clinical practice.

Conclusions

Even though major steps have been made in understanding the biology of IBD, we still have a long way to go before these findings can be implemented in clinical practice. Nevertheless, the gut microbiota holds great potential for clinical application, from diagnosing IBD to using it as therapeutic option. If we as a research community take the right steps, the implementation of the gut microbiota in the clinical practice of IBD is feasible in the foreseeable future.

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