The gut microbiota and inflammatory bowel disease

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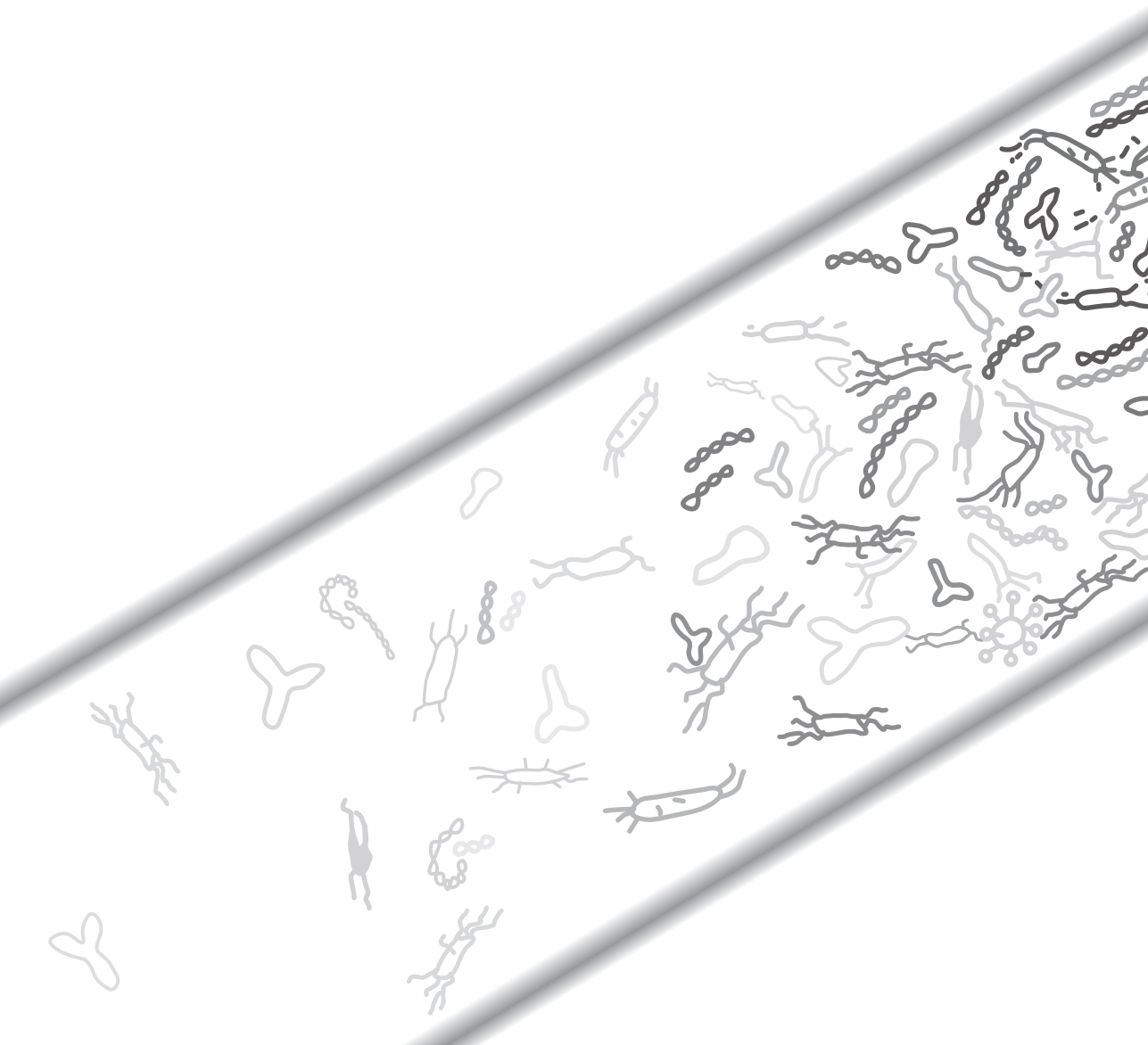
Introduction



Inflammatory bowel disease

Heterogeneous disorder

Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal (GI) tract in which patients experience periods of inflammation alternating with periods of remission. Crohn's disease (CD) and ulcerative colitis (UC) are the two main forms of IBD. In CD, inflammation can occur throughout the entire GI-tract and can be transmural, whereas in UC inflammation is mucosal and only located in the colon.[1,2] IBD has a prevalence ranging from 28.2 to 322 for CD and 43.1 to 412 for UC per 100.000 individuals in Western Europe.[3] The onset of IBD presents at a relatively young age, on average between 20 and 40 years of age.[1,2] The symptoms accompanying IBD include abdominal pain, diarrhoea, fatigue, rectal bleeding and weight loss. Patients can also develop extra-intestinal manifestation like arthritis, uveitis and spondyloarthritis or complications like colorectal carcinoma, fistulae and abscesses.[4] The disease course is very variable across patients with IBD; some patients regularly experience periods of inflammation with the accompanying symptoms, whilst others stay in remission for a long time and experience few symptoms throughout their disease course.[1,2] Taken together, all the differences observed in patients with IBD, including in disease location, disease course, occurrence of extra intestinal manifestations and complications, illustrate the heterogenous nature of IBD (Figure 1).

The golden standard for diagnosing IBD is performing an endoscopy in which intestinal biopsies are taken for histological assessment for the presence of microscopic inflammation.[5] If, however, no signs of IBD are detected via endoscopy, histological assessment or other tools used to make the diagnosis, IBD can be excluded, and the diagnosis of irritable bowel syndrome (IBS) is often made. IBS is a condition based on GI complaints in combination with alterations in bowel habits. It is the most commonly diagnosed disorder of the GI tract, although other causes for these symptoms should be excluded by procedures like endoscopies before an IBS diagnosis is made.[6] Considering that endoscopies are an invasive procedure for patients, a less invasive diagnostic tool is very much needed in patients with GI complaints.[7]

Figure 1. The heterogeneous nature of inflammatory bowel disease.

IBD management

The heterogeneous nature of IBD poses a major challenge in treating this disease. Collectively, drugs used for IBD management are aimed at 1) reducing inflammation during a disease exacerbation and 2) maintaining remission after induction therapy.[8] The management of IBD consists of drugs including 5-aminosalicyclic acid, corticosteroids, immunosuppressants like thiopurines, and biologicals like TNFα antibody therapies.[1,2] In recent years, more monoclonal antibodies have become available for treating IBD, for example the $\alpha 4\beta 7$ integrin inhibitor vedolizumab and the interleukin 12/interleukin 23 inhibitor ustekinumab. Moreover, small molecules have become available, such as the JAK inhibitor tofacitinib.[9] However, most of these treatments have significant side effects, are expensive and often prove to be ineffective.[10] While the initiation of biological therapies in IBD was at first reserved for a later stage in the disease course, more evidence is becoming available that supports starting biologicals earlier in the disease course in order to prevent irreversible damage of the intestine.[11] Current guidelines therefore state that early initiation of biologicals could be considered for patients who have an aggressive disease course or belong to the high-risk group.[10] Factors determining which patients belong to this high-risk group, however, have limited predictive power.[12,13] Besides drugs, surgical interventions are also part of IBD management. Half of patients (25%-30% UC, 70-75% CD) require surgical interventions during their disease course because of refractory disease, fibrostenotic disease, abscesses, fistulae, or the development of colorectal cancer.[14,15] In UC, this surgical intervention mostly consists of a colectomy, resulting in an ileostoma or an ileal pouch-anal anastomosis.[16] In CD, depending on the disease location, disease behaviour and extent of the affected intestine, the surgical intervention includes

resecting affected parts of the colon or small intestine.[17] Currently, very few clinical parameters or biomarkers are available that can predict how a patient's disease course will develop and/or how the patients will respond to specific treatments. Finding the optimal treatment for each individual IBD patient can therefore be challenging.[10]

Unravelling IBD pathogenesis

In order to improve the efficacy of therapeutic management of IBD, major efforts have been made to better understand the pathogenesis of IBD, which is still not fully understood. The current hypothesis is that IBD arises in an individual who is genetically susceptible and who has an inappropriate activation of the GI immune system. This immunological activation consists of, amongst other factors, an impaired epithelial barrier function, impaired autophagy and the production of pro-inflammatory cytokines. This is thought to occur in response to commensal bacteria or pathobionts that reside in the gut.[18,19] Host genetics has been studied by multiple genome-wide association studies, and these have identified more than 200 IBD risk loci, some of which are involved in the immune system.[20,21] These risk loci have also been shown to interact with microbes in the gut, with the *NOD2* or *CARD9* genes being two well-studied examples.[22,23] On top of that, environmental factors or the exposome, including the use of antibiotics, smoking and a Western diet, have been identified as risk factors for IBD development.[24,25] While none of these elements (genetics, environment or microbiota) can individually predict or explain the disease pathobiology, it is believed that the interaction of multiple factors is the potential cause of the disease.[26,27]

Studying the gut microbiota in inflammatory bowel disease

Gut microbiota in human health

In recent years, interest in studying the gut microbiota, i.e. all the microbes that collectively live in the GI tract, has grown tremendously.[28] More and more evidence has become available that the microbes residing in the gut form an entire ecosystem in which complex interactions between each of its members, as well as with the host and environmental factors, take place.[29] This ecosystem is involved in critical functions, including participating in the immune response, digesting the food we eat, metabolizing drugs, as well as many other functions.[30-32] Its implication in general health as well as in a broad range of chronic diseases, including cancer, metabolic disorders, psychiatric diseases, and inflammatory disorders like IBD have been widely studied.[26,27,33-37]

Gut microbiota research in IBD and its potential for clinical application

Before the start of my own scientific journey, multiple associations had been made

linking IBD with alterations in the gut microbiota composition. Mice administered with antibiotics or kept in germ-free conditions were, for example, significantly protected against the development of colitis.[38] Even though an IBD-specific signature of the gut microbiota had not yet been identified, IBD was known for having a decreased microbial richness in humans.[39,40] In addition, certain gut bacterial shifts have been identified to be associated with IBD, for example a decrease of *Faecalibacterium prausnitzii* and *Roseburia hominis*. Both these bacteria are butyrate producers and known to have anti-inflammatory properties.[40,41]

Even though these results had contributed significantly to our understanding of the pathogenesis of IBD, it had proven to be very difficult to translate these findings towards clinical practice. In other disorders, however, the potential of the gut microbiota for clinical application had been shown. One example is the treatment with the anti-cancer drug cyclophosphamide. Tumour-bearing mice that had been either treated with antibiotics or were germ-free had tumours that were resistant to this cancer treatment.[42] One of the first indications in which the gut microbiota itself was used as treatment – in the form of faecal microbiota transplantations – was in patients with bacterial overgrowth of *Clostridium difficile* after intensive antibiotics treatment.[43] Further exploring the gut microbiota composition in IBD, while also taking the heterogeneous nature of the disease into account, will help us to develop new diagnostic tools and treatment options and thereby facilitate clinical applications in IBD.

Development of gut microbial research

Our ability to study the gut microbiota to this wide an extent was made possible by the development of culture-independent sequencing techniques to characterize the gut microbiota. At first, microbial species were identified via culturing each microbe separately, which was very time-consuming and expensive.[28] Additionally, using this method, it was hard to identify microbes that are difficult to grow in a lab environment, for example anaerobes.[44] Through the development of 16S rRNA sequencing and shotgun metagenomic sequencing, two culture-independent techniques that were used to obtain the results described in this thesis, it became possible to study multiple microbes at the same time at a relatively low cost.[45,46] In 16S rRNA sequencing, the gene that encodes the small subunits of the prokaryotic ribosome is sequenced. This gene is present in most bacteria and archaea and consists of genetic regions that are variable and regions that are conserved. The combination of low mutation rates present in the conserved region and the higher mutation rates in the variable regions then allows us to characterize bacteria and archaea by using reference databases.[45] In metagenomic sequencing, the entire genetic content – not solely the 16S rRNA gene – is sequenced. Therefore, a higher resolution can be reached by identifying bacteria up to species and even strain level. By also analysing the sequenced microbial genes, more microbial

features can be identified, such as the functions of the microbial ecosystem, their implications in antibiotic resistance and mechanisms of virulence factors.[46-49] Given the rapid developments in the methods used to study the microbes in the gut, reaching a consensus about the appropriate terminology has been challenging.[50] In this thesis, the gut microbiota is defined as the presence of all microbes that are collectively residing the gut ecosystem, whereas the gut microbiome is defined as the collection of all microbial genetic content present in this ecosystem.[28,50]

The use of well-phenotyped cohorts

In the context of a heterogeneous disease like IBD, it is important to collect disease-specific phenotypes including disease location, the presence of inflammation and factors involved in IBD management, like certain medications and intestinal resections, which can potentially have an impact on the gut microbiota composition. On top of these factors, other host characteristics and life-style factors are also part of the patient/participant information that is important to capture when designing a microbiota study. Large population studies have shown the relation between these factors and the inter-individual variation in terms of gut microbiome composition.[26,27]

In this thesis, six cohorts were used for which extensive phenotypic data was available. First, we made use of two IBD cohorts, both from the University Medical Center Groningen (UMCG). One was a subset of the 1000IBD cohort for whom faecal samples were available (n=544). This cohort consists of patients with IBD from the UMCG, and the collection of biomaterials such as faecal samples and intestinal biopsies, clinical features and genetics was ongoing throughout the course of this thesis with the aim to collect this information for 1000 patients with IBD.[51] The second cohort consists of patients with IBD treated with vedolizumab (n=50) from whom faecal samples were collected prior to and 14 weeks after start vedolizumab treatment. Furthermore, we made use of a subset of the Lifelines cohort Lifelines DEEP (n=1539). Lifelines consists of 167,000 volunteers from the general population residing within the three Northern provinces of the Netherlands, for whom extensive phenotypic data was collected via questionnaires, including data on lifestyle, diet, medication use and diseases. In addition to the phenotypic data collected for Lifelines DEEP, there are also additional biomaterials available, such as blood and faecal samples. From these biomaterials, multiple 'omics levels were established, including metagenomics and host genetic data.[52,53] We also used the IBS cohort from Maastricht University Medical Center (n=336). This cohort consists of patients with IBS who were diagnosed by a gastroenterologist, meaning that they underwent extensive medical examinations to exclude other causes of their GI complaints. These patients were grouped with age- and sex-matched controls without GI complaints.[54] Finally, we made use of two Dutch cohorts from the Radboud University Medical Center Nijmegen. The first, 500FG, consists of healthy volunteers (n=534).[55] The second, 300OB, consists of individuals with

a BMI higher than 27 (n=302) in whom the presence of obesity-related comorbidities has been assessed.[56]

This thesis - Aims and outline

The aim of my scientific work was to explore the role of the gut microbiota in the context of gastrointestinal disorders and, more specifically, in IBD. Furthermore, considering my medical background, I aimed to translate these findings towards clinical practice by using genetic information and gut microbial findings in IBD management.

Part I - Exploring the role of the gut microbiota in IBD

The first part of this thesis aims to gain understanding of IBD by exploring the role of the gut microbiota in the disease. The work presented in **chapter 2** shows the alterations in the gut microbiota associated with IBD. By using the metagenomes derived from faecal samples, I show differences in the microbial functions, virulence factors and antibiotic resistance in IBD compared to the general population and patients with IBS. Furthermore, I describe the potential for clinical application by showing that the gut microbiota can be used as a diagnostic tool to distinguish between IBD and IBS. To gain insight into how the microbes identified in **chapter 2** potentially interact with the other members of the gut microbiota in its ecosystem, I constructed microbial co-abundance networks in **chapter 3**. In **chapters 4** and **5,** specific questions are addressed concerning IBD-specific factors. Since most gut microbiota studies are based on faecal samples, reflecting the colonic microbiota, and the small intestine plays an important role in the host, in **chapter 4** I aimed to explore the small intestinal microbiota. Here I used the metagenomes of faecal samples collected from patients with IBD with an ileostomy or ileoanal pouch representing the small intestinal microbiota. The metagenomes of these patients were compared to the metagenomes of the general population and the remaining patients with IBD, with or without having intestinal resections prior to faecal sample collection. Considering the importance of validating already identified gut microbiota findings, in **chapter 5** I aimed to replicate the interaction of the *SLC39A8* missense variant and the gut microbiota by using 16S rRNA sequencing data from patients with IBD and healthy controls from Lifelines-DEEP.

Part II: Clinical translation – drugs and (meta)genomics

In the second part of the thesis, the focus shifts towards the clinical translation of the findings, in particular towards the relation of genetics and the gut microbiota to drugs in patients with IBD. Major improvements can be made in order to make drug development more efficient in IBD. In **chapter 6**, we show how we could use current knowledge on genetic information in IBD to identify new drug targets or to reposition existing drugs for

the IBD diagnosis. In **chapter 7** we focused on the IBD drug vedolizumab, by collecting the faecal samples before and after start of the treatment in order to study the role of the gut microbiota in treatment response of vedolizumab in IBD. In this study, we also assessed the role of 92 proteins determined from the serum samples of these patients in order to predict treatment response. Under the hypothesis that drugs show interactions with the gut microbiota, in **chapter 8** we studied the impact of 41 commonly used drugs on the gut microbiota composition. For this, we used the general population cohort as our IBD cohort, in which comorbidities and polypharmacy are more common. In **chapter 9**, we describe our vision and future perspectives on how to improve the study of the gut microbiota in IBD and how these improvements lead towards clinical application of the findings. These include the use of the gut microbiota as a diagnostic tool, a predictor of treatment response and in IBD treatment. In **chapter 10**, I provide an overview of the main findings of this thesis, their impact on the field and their limitations. Lastly, I discuss my view on how the field should progress and how we could use the gut microbiota in IBD clinical practice.

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PART I

Exploring the role of the gut microbiota in IBD