Understanding the Complexity of Inflammatory Bowel Disease from a Multi-Omics Perspective
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Chapter 4

IBD genetics and the gut microbiome

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Abstract

The pathogenesis of inflammatory bowel disease (IBD) is determined by multiple genetic variants and by gut microbiota. The role of interactions between these two factors has emerged recently. In this chapter, we focus on the crosstalk of gut microbiota and IBD genetic risk factors and give a review on the current progress and perspective of this research field.

Introduction

Recent decades have witnessed exciting progress in unravelling the pathogenic background of inflammatory bowel disease (IBD). It is now broadly understood that the disease results from a disturbed state of the mucosal immunity in genetically susceptible individuals. However, it is now also recognized that these genetic factors are not the sole contributors to disease development; they act in synergy with the external and internal environment, in particular with the gut microbiota. Inherent genetic factors determine the physical and chemical setup of the host intestinal environment and mucosal immunity, and this occurs in communication with the gut microbiota. Looking at this from the gut microbiome perspective, the microbial community is involved in metabolic pathways and in training the host immune system. There is also mounting evidence for cross-talk between host genetics and the gut microbiota. In this chapter we summarize current knowledge of the genetic factors involved in IBD and how they modulate the predisposition to disease through interaction with gut microbiota.

Genetics of IBD

Research on IBD genetics has been evolving throughout the eras of twin-studies, family-based linkage disequilibrium mapping and genome wide association studies (GWAS). The heritability of IBD was first observed by epidemiological research in large twin-studies from Swedish, UK, and Danish populations, which showed higher concordance between monozygotic pairs than dizygotic pairs for both ulcerative colitis (UC) and Crohn's disease (CD). The landmark discovery of this era was the association of the NOD2 gene to CD through linkage disequilibrium mapping.

The first GWAS on IBD was carried out in Japanese patients with CD and healthy controls. Although it had a relatively small sample size (n=94 CD patients) and a limited number of single nucleotide polymorphism (SNP) markers (n=70,000), the study identified that TNFSF15 showed association with increased risk of CD. To date, there are now more than 240 genomic loci that have been associated to IBD. A meta-analysis of IBD data from 15 GWAS identified 163 independent loci, including 30 CD-specific loci, 23 UC-specific loci, and 110 loci thought to play key parts in both. A recent study compromising more than 20,000 individuals that looked at published summary statistics found more than 240 IBD risk loci. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) IBD Genetics Consortium (IBDGC) is now focusing on whole exome variants from 7,500 patients with UC, 7,500 patients with CD and 5,000 controls from all over the
world to assess the protein coding and rare mutations affecting the disease. Overall, all the discoveries to date point to genetics playing an important role in IBD, with currently identified genetic variants explaining more than 13.1% of the variance in CD disease susceptibility and 8.2% of the variance in UC disease susceptibility.

The human gut microbiome

Methods for host gut microbiome analysis

More than $10^{14}$ micro-organisms populate the adult gastrointestinal tract, an ecosystem consisting of bacteria, archaea, eukaryotes, and viruses. Gut microbial traits–taxonomy, functional pathway or community diversity–can now be efficiently quantified by sequencing technology. The two major sequencing methods currently used for microbial quantification are 16S rRNA gene sequencing and metagenomics sequencing. 16S rRNA sequencing is a targeted sequencing method enriching only the highly diverse sequence of the 16S ribosomal RNA gene region in order to identify taxa based on a genome database of known microbiota signatures. Metagenomics sequencing is a more precise technique that captures the genomic signature of all genes from all organisms present in the sample material, thus providing researchers a comprehensive overview of both known microbes and unknown microorganisms. Metagenomics sequencing not only goes deep into the strain level of bacteria, it is also able to identify bacterial metabolic gene functions. Other sequencing methods have been developed for specific annotation of the fungal community and virome-specific analysis, but only a few individual studies have applied these to IBD thus far.

Factors influencing host gut microbiota

The gut microbiome can be affected by many factors over the course of an individual’s life, with rapid changes occurring in the first years of life. The initial gut ecosystem in a baby’s early life is affected by maternal factors, while during the first year of life, the maturation process is characterized by an increase in the richness and diversity of the bacterial community. In later adulthood, the human gut microbiome is more modulated by other factors, including medication-use, diet, smoking, and mucosal immunity.

Dysbiosis of intestinal flora in IBD

Over a long period of evolution, the human intestinal flora has formed a mutual interdependence and mutual benefit micro-ecosystem with its host. The normal condition of the intestinal mucosal immune system is therefore usually a state of immune tolerance for the gut flora, a condition termed homeostasis. Dysbiosis takes place when this normal composition of gut microbiota is impaired. In IBD, it is now well recognized that an increase in phyla Proteobacteria and a decrease in phyla Firmicutes are observed in IBD patients as compared to healthy controls. In CD Escherichia coli (belonging to family Enterobacteriaceae) is enriched, while Faecalibacterium prausnitzii (belonging to family Clostridiaceae) is relatively depleted. Interestingly, bacteria from phylum Bacteroidetes has been reported to show either decrease or increase in IBD. The alteration of taxa also leads to alterations in taxa-related microbial functions. For example, the level of short-chain fatty acids (SCFAs), a basic energy source for colonic epithelial cells, is lower in IBD, a shift that could be explained by lower abundances of SCFA-producing bacteria like Faecalibacterium and Roseburia. In addition to shifts in bacterial taxa and functions, IBD is also characterized by changes at the community-level: the richness and diversity of the gut microbiota has been observed to be significantly decreased in IBD.

Host–microbiota interactions

In general, direct interactions between host and intestinal microbiota take place via the mucosal physical barrier and innate and adaptive immune response. For example, the intestinal epithelial cell compromises the first physical barrier to gut microbiota, where secretory immunoglobulin A (IgA) and antimicrobial peptides are concentrated in the outermost layer. IgA helps commensal bacteria to anchor, survive and reproduce on the intestinal epithelial surface, as well as playing a role in combatting pathogens. An IgA-deficient gut shows a decline in anti-inflammatory bacteria and an expansion of pro-inflammatory bacteria. The initial recognition of pathogens is carried out by host pattern recognition receptors, such as Toll-like receptors, that enrol and activate a line of downstream immune cells, thus contributing to innate and adaptive immune processes. Various mouse model experiments have demonstrated that modulation of gut microbiota could alter immune cell production, leading to a different susceptibility to inflammation or other abnormal symptoms.

Recently, the human gut microbiota has been shown to be dependent on host genetics, as established by heritability calculations from twins and isolated and kinship populations, although the estimated heritability of taxa varies from 1.9% to 8.1%. A Swedish twins-study in both healthy and CD individuals revealed that the bacterial similarity is more consistent between healthy twins than between twins with CD, which suggests there is an essential interaction between host IBD genetics and the gut microbiota.
Chapter 4 - IBD genetics and the gut microbiome

The sections below review what is currently known about the genetics-microbiome interaction in the context of known IBD-risk genes from different study levels: candidate gene studies and microbiome GWAS.

IBD genetics and gut microbiome interaction based on candidate gene studies

Once it became evident that host genetics can change susceptibility to IBD, candidate IBD-risk genes became a focus of research looking into their role in gut microbiota alteration.

Nucleotide Binding Oligomerization Domain Containing 2 (NOD2)

The first-identified IBD-related gene, NOD2, was discovered to play a decisive role in regulating the intestinal microbiota both in innate and adaptive immunity^{50,51}. This gene belongs to the NOD-like receptor family that is responsible for binding muramyl dipeptide (MDP) to the bacteria^{52}. By sequencing the faecal and ileal samples from Nod2-deficient and wild type (wt) mice, it was found that the relative abundance of Bacteroidetes was significantly increased in absence of Nod2^{25}. The impaired intestinal microbe community caused by loss of functional Nod2 was then confirmed by several independent mouse experiments^{54-56}. In addition, studies have also shown further evidence of the connection between gut microbial alteration and disease predisposition. Interestingly, Nod2-deficiency is linked to increased levels of Bacteroides and Lachnobacterium^{57}, both considered to be pathogenic bacteria in IBD^{58,59}, which leads to development of histological colitis as well as tumour growth^{57,58}. Another mouse study further proved that Nod2 deletion leads to metabolic dysfunction; increased immune-gene expression—including expression of T cell activation receptors like Cdg, Cdg, and Cdg5 and chemokines like Ccl2 and Ccl5—was found in Nod2 deletion mice, accompanied by higher levels of serum cholesterol and triglycerides and an increased abundance of Bacteroidetes^{60}. In addition, in contrast to Nod2-mutant animals, Nod2-wt animals are protected from intestinal inflammation through normal regulation and maintenance of microbial hemostasis in the GI tract^{51}. Correlations between NOD2 polymorphisms and the relative abundance of bacterial taxa in the gut were also identified in human studies. Based on 16s rRNA gene sequencing of mucosal biopsies from three independent IBD cohorts from the USA, Canada and the Netherlands (comprising 474 individuals in total), an increased percentage of Enterobacteriaceae was associated with NOD2 risk allele counts^{62}. Therefore, it is clear that NOD2 is essential for gut microbiota that mediate the mucosal immune response, metabolic levels and the occurrence of inflammation.

Autophagy Related 16 Like 1 (ATG16L1)

Another established IBD risk gene is ATG16L1 with a coding SNP (T300A), which has been suggested to be associated with CD by two large-scale GWAS^{63,64}. ATG16L1 is considered to participate in cell autophagy, not only affecting the cell-cycle and cell-proliferation, but also regulating the development of CD4 and CD8 T-cells and autophagy-dependent immune response^{65}. A severe decline in CD4 T cells was observed in mice with an Atg16l1 deletion, resulting in spontaneous intestinal inflammation^{66}, and autophagy activation to MDP was blocked in human epithelial cell lines carrying the ATG16L1 T300A variant^{67}. Another study used different stimulators of NOD2 in different ATG16L1 polymorphism genotypes from peripheral blood mononuclear cells in healthy individuals and patients with CD. Higher levels of the pro-inflammatory cytokines interleukin 1β (IL-1) and IL-6 were produced in carriers of the T300A risk variant, which suggested an interaction between ATG16L1 and NOD2 in autophagy in the context of IBD^{68}. Consistent with the finding from human cells, a similar immune response, including decreased antibacterial autophagy and increased IL-1 production, was also observed in a later T300A knock-in mouse experiment^{69}. Direct evidence for ATG16L1-related impaired autophagy lacking a defence against specific pathogens comes from a Dutch CD patient study^{70} in which Fusobacteriaceae, Bacteroidaceae and Enterobacteriaceae were significantly enriched in inflamed ileal tissue from patients homozygous for ATG16L1-T300A as compared to tissue from non-mutated patients. In addition, monocytes homoygous for the ATG16L1 risk allele also lost the ability to eliminate adherent-invasive Escherichia coli. Therefore, the ATG16L1 risk mutation causes an ineffective autophagy-based immune response to gut pathogens in IBD.

Fucosyltransferase 2 (FUT2)

Given that mucosal bacteria interact with their host directly through the intestinal mucosal barrier, chemical secretions from mucosal cells are key factors for influencing the gut microbiota. One of these secretions is glycan, which facilitates the adherence of particular taxa to the outer layer of mucus^{71,72}. The FUT2 gene is responsible for transferring terminal fucose residues and mediating the interaction of the gastric epithelium with intestinal microbiota. Homozygous individuals lacking functional FUT2 alleles, who are
also defined as “nonsecretors” since they do not express ABH blood group antigens on mucosal secretions and surfaces, show a higher susceptibility to CD and to alteration of gut microbiota. A decrease in microbial diversity and changes in several taxa were observed in nonsecretors from both human population studies and mouse experiments. A recent study reported that the lower abundance of Blautia in nonsecretors was mediated by a low level of epithelial cell adhesion molecules (Ep-CAM). This indicates that loss of function of FUT2 leads to an abnormal mucosal barrier, followed by a change in gut microbial community and susceptibility to CD.

Caspase Recruitment Domain Family Member 9 (CARD9)
The risk association of CARD9 to CD and UC was proven by a case-control study in a large Dutch cohort. In Card9 knock-out mice, reduced levels of several immune cytokines and more intestinal fungi were observed as compared with wt mice. After Citrobacter rodentium infection, higher susceptibility was also found in Card9-/- mice. All of these suggest CARD9 is essential for immune response to intestinal pathogens.

Other IBD risk genes
Several other IBD-related genes have also been found to influence the composition and diversity of gut microbiota. Colonization with Escherichia coli and Bacteroides vulgatus was observed to prevent IBD development in mice deficient in Il-2, which codes for a cytokine expressed in intestinal tissue. Loss of the NLRP12 gene in humans also led to lower levels of the protective gut commensal bacteria Lachnospiraceae and Erysipelotrichaceae, leading to an inflammatory response. Most IBD-related genes interact with gut microbiota through the innate or adaptive immune response, as proven by mouse experiments, cell experiments or candidate gene testing in the human population. Candidate genes studies in mouse and cell systems are a good way to focus on specific genes that control other factors, thus allowing a better understanding of the immunological mechanisms that underlie the observed association between disease, microbiota and genotype. In particular, these experiments can avoid the confounding effects of factors like diet, drug usage and lifestyle, and help provide straightforward and strong evidence for the mechanistic hypothesis. However, candidate gene methods largely depend on previous knowledge and lab work and are less able to provide a comprehensive view of the interaction between host genetics and the gut microbiota.

Table 1 Interactions between IBD risk genes and bacterial traits. We showed examples of genes involved in both IBD and microbiome genetic susceptibility, categorized by immune response types and study method types.

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Microbial Traits</th>
<th>Study Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL10</td>
<td>GO:0031461 cullin-RING ubiquitin ligase complex Enterobacteriaceae</td>
<td>both microbiome GWAS and candidate studies</td>
<td>81,82</td>
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<tr>
<td>IL2</td>
<td>Escherichia coli, et al.</td>
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<tr>
<td>ATG16L1</td>
<td>Fusobacteriaceae, Bacteroidaceae and Enterobacteriaceae</td>
<td>candidate genes study</td>
<td>70</td>
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<tr>
<td>BANK1</td>
<td>Beta-diversity</td>
<td>microbiome GWAS</td>
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<td>EFR3B</td>
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<td>microbiome GWAS</td>
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<td>Beta-diversity</td>
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<td>SH2D4B</td>
<td>YS2/4Cd-2</td>
<td>microbiome GWAS</td>
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<td>CCL2/CCL7/CCL11</td>
<td>PWY-6654 phosphopantothenate biosynthesis III</td>
<td>microbiome GWAS</td>
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<td>CD5/CD6</td>
<td>GO:0009231 riboflavin biosynthetic process Bacteroidales PWY-5154 L-arginine biosynthesis III</td>
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<td>TNFSF15</td>
<td>Prevotella, et al.</td>
<td>both microbiome GWAS and candidate studies</td>
<td>87,62</td>
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<tr>
<td>CARD9</td>
<td>Citrobacter rodentium, fungi</td>
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<td>NOD2</td>
<td>Enterobacteriaceae, Bacteroidetes</td>
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<td>53,62,82</td>
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<td>FUT2</td>
<td>Blautia</td>
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<td>IL23R</td>
<td>PWY-6215 4-chlorobenzoate degradation</td>
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<td>DAP</td>
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<td>Lachnospiraceae, Erysipelotrichaceae</td>
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<tr>
<td>SLC39A8</td>
<td>Roseburia et al.</td>
<td>candidate gene study</td>
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</table>
IBD genetics and gut microbiome interaction based on microbiome GWAS

While previous data came from candidate genes, data from genome-wide genotyping in humans and the gut microbiome now allow us to perform microbiome GWAS\(^9\). Several milestones have now been reached in large-scale cohorts that have extended our knowledge about the relation between IBD genetic factors and gut microbiota.

The Human Microbiome Project (HMP) was launched from 2008 with the aim of characterizing the complexity of microbial communities at different sites in the human body in order to better understand how their metabolic functions contribute to a variety of human phenotypes\(^9\). In 2015, the first association analysis was carried out between host genome variation and microbiome composition at 10 body sites using 93 individuals from the HMP\(^4\). 83 SNPs enriched in immune-related pathways were associated with microbial taxa, including genetic variants in IL2 and CXCR4 genes. In another study performed in 60 Hutteries (a Canadian founder population), several bacterial taxa were found to be influenced by host genetics\(^9\), including the genus Akkermansia by gene PLD1, a relationship that had been established in an earlier mouse study\(^9\). Through enrichment analysis, microbial quantitative trait loci (mbQTL) in this study were also found to be concentrated at immune processes. In 2016, the heritability of 945 bacterial taxa were estimated in 1,126 UK twins, with multiple bacteria from Firmicutes, Actinobacteria, Tenericutes, and Euryarchaeota phyla found to be heritable\(^9\). By testing a SNP set related to IBD and heritable taxa, GNA12, which codes for a guanine nucleotide-binding protein involved in Rho signalling pathway regulating cytokine production\(^9\), was associated with an increased abundance of Clostridiaceae. GNA12 is important for intestinal innate immune response and is associated with CD\(^9\). In addition to bacterial taxa, three variants were correlated to microbial beta-diversity, which described the similarities in microbial composition between individuals.

Three large-cohort mbQTL studies performed in 2016 revealed the more general interaction between host genetics and gut microbiota. Using metagenomics sequencing for faecal bacteria detection and Immunochip and CoreExome chip for human genomic variant genotyping, a discovery-replication meta-analysis was performed on three Dutch cohorts (totally 1,154 individuals) between population genotype and bacterial taxa, metabolic MetaCyc pathway and Gene Ontology (GO) terms\(^9\). At the whole genome-wide level, nine genomic loci were associated with microbial taxonomies and 33 genomic loci with bacterial pathways and GO terms. Most of the genetic loci were related to complex diseases and innate and adaptive immunity. Strikingly, several IBD-risk mutations were also identified: CCL2, chemokine (C-C motif) ligand 2, a kind of cytokine, participates in recruiting immune cells for virus detecting\(^9\) and anti-inflammation\(^9\); IL-10, another cytokine, is involved in pathogen-induced inflammation disease\(^9\); DAP, which playing a role in the molecular organization, regulates the autophagy-dependent immune response\(^9\); interleukin 23 receptor (IL23R) produces signalling protein in T cell and monocyte membrane\(^9\); and genes from C-type lectin molecules (including CLEC6A, CLEC4E, CLEC7A and CLEC4G) were found to have critical importance for anti-bacterial immune reaction\(^9,9\). In addition, this study also confirmed the association of NOD1 and NOD2 genes with MetaCyc pathways or GO terms driven by Enterobacteria, showing agreement with previous candidate genes studies.

In a Canadian cohort study based on Immunochip and 16s sequencing\(^9,4\), 58 host loci were found to influence the gut microbiome taxa with four loci replicated in other geographically different cohorts (American and Israeli) together compromising 1,516 individuals. Among the four replicable loci, the UBR3 gene was annotated to an immune-related pathway which is responsible for external pathogen recognition and defense\(^9\). In a study by Wang et al\(^9\), more than 2,000 individuals were included for microarray genotyping and 16s sequencing, and 42 loci were found to be correlated to beta-diversity and 40 loci to bacterial taxonomies. Notably, the C-type lectin related gene, CLEC16A, was again identified to be associated with gut bacterial community. The most interesting finding here is that the gene VDR (vitamin D receptor) affected both microbial beta-diversity and Parabacteroides. Vitamin D is vital for immune response, including activation of monocytes, regulation dendritic cell maturation and production of cytokines in T cells\(^9,10\). Vdr deficient mice showed alteration of beta-diversity, and upregulation of VDR in CD patients showed a decrease in Parabacteroides that caused severe inflammation\(^9\). Large cohort mbQTL studies thus further support that there is cross-talk between IBD-related genes and the gut microbiome that occurs mainly through the immune response. More large-scale studies of the genetics of the gut microbiome are on the way\(^9\) and will likely identify more genetic variants associated to the microbiome composition.
Greater sample sizes for IBD and microbiome GWAS to handle the power issue

Genetic association studies of IBD have now resulted in more than 240 loci being associated with the disease. Altogether, these loci can explain a substantial proportion of the heritability of CD and UC, but not all of it. Despite the fact that some fraction of this missing heritability must come from complex, non-additive interactions of genetics with the microbiome and components of the external environment, we expect more risk loci to be identified in the near future with increases in sample size, which will increase discovery power, and the inclusion of new populations from different ethnicities.

Larger, well phenotyped IBD cohorts to study within-disease heterogeneity

GWAS of microbiome composition in cohorts of IBD patients are particularly important. We already know that an IBD microbiome is substantially different from a healthy one and is characterized by a major decrease in normal flora (including decreases in beneficial microbes such as Blautia, Faecalibacterium, Roseburia) and an increase in known pathogenic bacteria (including Escherichia, Shigella and other members of Enterobacteriaceae family). This shift may reveal novel genetic mechanisms that drive microbiome composition and functionality. The first study on this subject, while limited by small sample size and low-resolution 16S-based microbiome profiling, did reveal IBD-specific mbQTLs that were not found in healthy subjects. More specifically, it revealed SNPs in the NOD2 locus that were associated with an increase in potentially pathogenic microbes of Enterobacteriaceae family. Interestingly, it was later shown in healthy subjects that this locus also acts as an mbQTL for enterobactin biosynthesis, mostly predicted from Escherichia coli. We believe the further studies that tell us more about this kind of heterogeneity will allow to understand more about gene-microbiome interaction in relation to IBD.

Conclusions and perspective

The complexity of the genetic architecture of IBD underlies the high diversity of potential mechanisms that could translate inherited genetic makeup into a clinical manifestation. Despite several established mechanisms of gene-microbiome crosstalk that lead to a disease, our systematic knowledge on this subject remains very limited. However, as we discuss below, there are a number of logical next steps that researchers will make to shed more light on the mechanisms of IBD development.
adding it to the analysis model will improve the power of genetics studies. We do expect to benefit from further studies on the interaction between genetics and microbiome that shape this heterogeneity, with well-collected phenotype and environmental factors cohorts.

From research side to clinical side
Examining all the known mbQTL studies, we noticed that only a small number of mbQTLs overlapped with IBD-risk genomic regions. Therefore, post-mbQTL study is necessary for the coming IBD research.

So far, limited functional studies have been carried out to explore the biological meaning behind the statistical associations. NOD2 and IL-10 are two of the few well-studied IBD-related mbQTL, as the former is involved in the NOD-receptor signalling pathway in innate immune response and the latter acts as an inhibitor of inflammation. Intestinal inflammation was observed in knockout mouse experiments for both of these two genes. Antibiotic medication and faecal microbiota transplantation treatment have now been widely used on IBD patients, however, the molecular mechanisms underlying a large number of IBD risk loci, and how they influence the gut microbiota, remain unknown. Therefore, experiments and clinical trials should be integrated to give better guidance for IBD clinical application.

Another gap that needs to be addressed in the future is that current studies primarily collect samples from IBD patients with established clinical symptoms, even though the disease is probably triggered years before clinical determination. There is still lack of knowledge of microbial change during the whole disease process. Therefore, the causality or consequence of gut microbial change to IBD is still unclear. Dissecting the different clinical periods of IBD is a key to understand its pathogenesis, and a pre-clinical IBD study in Dutch twins is currently ongoing. Combining multiple IBD factors, including the gut microbiota, at a series of time points between cohorts of IBD-discordant, IBD-concordant and non-IBD-concordant twins, should provide new insights into the mechanism behind the cross-talk between IBD and multiple factors that are meaningful for clinical prevention and therapy.

In-depth characterization of IBD genetics and gut microbiota
The majority of IBD candidate genomic loci identified so far are non-coding variants, which complicates causal discovery. Fine-mapping approaches through high-resolution genotyping and exome-wide sequencing mainly focusing only on protein-coding loci can implicate variants at likely IBD- causative genes. And now the similar strategies should be applied in microbial GWAS. More than 95% of known mbQTLs are located in intronic or intergenic regions. Only several clear genes harbouring variants, such as FUT2, can cause amino acid change during protein generation. In addition, high-resolution microbial sequencing allows us to go deeper into the species or even strain level of gut microbiota. Therefore, in-depth characterizing of IBD-related genome regions and gut microbiota should be considered in the future.

In conclusion, this chapter summarizes what we know about how genetics can modulate human IBD susceptibility via intestinal immunity and the gut microbiota and lays out how our understanding of the influence of cross-talk between IBD genetics and gut microbiota is still in an exploratory stage. However, even with the challenges we have discussed, we believe this field holds promise for discovering new ways to future IBD precision medicine and treatment.
References


