Determinants of IBD Heritability: Genes, Bugs, and More

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Defining the etiology of inflammatory bowel disease (IBD) continues to elude researchers, in part due to the possibility that there may be different triggers for a spectrum of disease phenotypes that are currently classified as either Crohn's disease (CD) or ulcerative colitis (UC). What is clear is that genetic susceptibility plays an important role in the development of IBD, and large genome-wide association studies using case-control approaches have identified more than 230 risk alleles. Many of these identified risk alleles are located in a variety of genes important in host-microbiome interactions. In spite of these major advances, the mechanisms behind the genetic influence on disease development remain unknown. In addition, the identified genetic risks have thus far failed to fully define the hereditability of IBD. Host genetics influence host interactions with the gut microbiota in maintaining health through a balance of regulated immune responses and coordinated microbial composition and function. What remains to be defined is how alterations in these interactions can lead to disease. The nature and cause of changes in the microbiota in patients with IBD are poorly understood. In spite of the large catalog of alterations in the microbiota of IBD patients, inflammation itself can alter the microbiota, leaving open the question of which is cause or effect. The composition and function of the gut microbiota are influenced by many factors, including environmental factors, dietary factors, and, as recent studies have shown, host genetic makeup. More than 200 loci have shown potential to influence the microbiota, but replication and larger studies are still required to validate these findings. It would seem reasonable to consider the combination of both host genetic makeup and the inheritance of the microbiota as interdependent heritable forces that could explain the nature of an individual's susceptibility to IBD or indeed the actual cause of IBD. In this review, we will consider the contribution of the host genetics, the microbiome, and the influence of host genetics on the microbiota to the heritability of IBD.

Key Words: human microbiota, dysbiosis, GWAS, inflammation, epigenetics

INTRODUCTION

The factors that trigger the onset of inflammatory bowel disease (IBD) are unknown; however, the current hypothesis proposes that genetically susceptible individuals experience an environmental trigger resulting in an inappropriate immune response potentially against certain gut microbes.[1, 2] The term "heritability" is commonly used to refer to the observed differences in a trait among individuals of a population that are due to genetic differences or inheritance from previous generations. In addition to genetic inheritance, factors including environment and diet can contribute to the variation between individuals and may even contribute to clustering of traits within families, and therefore suggest a hereditable influence. It is clear from human studies that there is a strong heritable component to IBD, but the genetic basis for this does not seem to fully explain the observed heritability.[3, 4] As we begin to appreciate the host interactions with the microbes that occupy the gut,[4–6] we have also seen elements of hereditability influencing the composition of gut microbiota. In mouse work, this appreciation has led to the importance of using littermate controls to assess genetic contribution to a phenotype.[7] In this review, we will discuss the notion that hereditability of IBD is a function of not only of host genetics, but also of the gut microbiome and other factors that influence their interaction.

GENETICS OF IBD

It is well established that IBD susceptibility has a strong genetic component,[8] with up to 12% of IBD patients having a family history of IBD.[9] Family and twins studies have shown that the degree of heritability of IBD depends on the disease phenotype, with host genetics playing a smaller contribution in ulcerative colitis (UC) as compared with Crohn's disease (CD).[4] First-degree relatives (FDRs) of IBD patients have increased risk of IBD, and this risk is higher in CD relatives compared with UC relatives.[9] The incidence rate ratio in CD FDRs is 7.77 (95% confidence interval [CI], 7.05–8.56), whereas the incidence rate ratio in UC FDRs is 4.08 (95% CI, 3.81–4.38).[9] Furthermore, the concordance rate in monozygotic twins for UC is 10%–15%, whereas it reaches 30%–35% in CD.[10, 11]

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To date, genome-wide association studies (GWAS) have identified more than 230 single nucleotide polymorphisms (SNPs) associated with IBD.[3, 12, 13] For many of these SNPs, the risk ascribed (as expressed by the observed odd ratios [ORs]) remains relatively low, especially for most of the newly discovered SNPs.[3] For instance, the first risk variant for IBD was located in the nucleotide oligomerization domain containing protein 2 gene (*NOD2*), which has the highest OR of 3.1 in CD. Additionally, the interleukin 23 receptor (*IL23R*) risk allele has an OR of 2.0 in IBD, whereas recently identified rs1748195 located in the *DOCK7* gene only has an OR of 1.07 in European descent.[3, 4]

In order to assess the combined effect of all the risk alleles, a genetic risk score (GRS) can be calculated using the observed odds ratios for each SNP and disease phenotype. Healthy FDRs of CD have a higher CD-GRS (calculated using only SNPs associated with CD) than the general healthy population, but their CD-GRS is lower than individuals diagnosed with CD.[14] Similarly, FDRs of UC have a higher UC-GRS than the general healthy population. It is also important to acknowledge that there are many instances in which a patient with CD may have a family member with UC and vice versa.[9] In 1 study, 20.5% of families with 2 or more affected members were pure CD families and 43.4% were pure UC families, whereas 36.1% were mixed CD and UC families.[9] Perhaps this is not surprising given that there are many genetic loci that are shared between CD and UC.[4] Therefore, there is a clear genetic basis to the observed increase of IBD in families; however, the IBD risk–associated SNPs seem to account for only a portion of the observed heritability of IBD. Conversely, many healthy individuals who carry these risk alleles never develop disease.[4]

Influence of Ethnicity on Genetic Associations of IBD

Ethnic groups for which IBD incidence has historically been rare have recently been experiencing a sudden rise in the number of new IBD cases.[15] Several GWAS were performed in defined ethnic populations,[16–18] including Japanese, Koreans, Ashkenazi Jews, etc. These studies describe small but noticeable differences in the list of SNPs that are associated with IBD in different ethnicities as compared with Caucasians of European decent (the most widely studied group affected by IBD). Some loci such as *NOD2* seem to be more significantly associated with IBD in certain cohorts/populations. For example, the *NOD2* locus has a smaller effect size in East Asian population[3] as compared with the European population. In more recent work looking at the Japanese population, 2 East Asia–specific IBD susceptibility loci were identified,[19] and in a Korean cohort, 3 novel CD risk loci were identified.[17] In 2012, Jostins et al. identified 163 SNPs associated with IBD in cohorts of European ancestry, of which 30 were specific to CD, 23 to UC, and 110 were shared between both.[4] Among those SNPs, 41 were not replicated in a separate non-European cohort.[3] In another independent GWAS study with 25,305 individuals of European ancestry, almost all genetic risk loci were shared across different ethnic populations.[12] Lastly, in a large study of Ashkenazi Jews, the most common IBD-associated variants conserved the directionality of their effects, as observed in the European ancestry cohort.[16] Overall, these GWAS studies suggest that a large common core of IBD risk–associated SNPs exists, which may help explain similar pathophysiology across ethnicities.

Genetically Defined Pathways Associated With IBD

More than 230 IBD risk alleles have been identified, suggesting a number of genetic pathways involved in the observed risk. The strongest genetic risk locus in IBD is *NOD2*,[20–22] which codes for an important cytosolic pattern recognition receptor in the host-microbe immune response. *NOD2* is expressed in gut epithelial cells (including Paneth cells) and *lamina propria* lymphocytes (including T cells) but is most strongly expressed in monocytes and macrophages.[23–26] This receptor recognizes and binds to muramyl dipeptide (MDP), a component of peptidoglycan derived from the bacterial cell wall.[27] Binding of MDP to NOD2 results in oligomerization and induces activation of NK-κB and MAPK and increases transcription of pro-inflammatory cytokines.[7, 28] The 3 common *NOD2* mutations observed in IBD are located within the leucine-rich repeat (LRR) domain responsible for binding MDP[29]; suggesting that the absence of bacterial sensing via NOD2 could explain the increased risk.[30] Paneth cell antimicrobial response abnormalities have also been associated with *NOD2* mutations.[31, 32] Paneth cells secrete antimicrobial peptides such as α-defensins, which are reduced in IBD patients,[33] perhaps related to the abnormal Paneth cell morphology that has been observed in CD patients with *NOD2* mutations.[34] Furthermore, abnormal Paneth cells have been associated with an altered ileal microbiota in pediatric CD patients.[35] Taken together, this suggests that the role NOD2 plays in IBD pathogenesis is via influencing responses to gut microbiota, although the exact mechanism(s) by which this occurs has not yet been defined.[7]

Autophagy, a degradation system used by cells for clearance of cytosolic debris and dysfunctional organelles, and possibly in the intracellular responses to pathogens, has also been implicated in IBD. Genetic variants identified in several genes involved in the pathway, including *ATG16L1, LRRK2,* and *IRGM* have been identified as risk-associated loci for IBD.[36–38] The function of the autophagy pathway in general cell maintenance and its role in response to intracellular microbes suggest diverse mechanisms that can lead to IBD, although it remains unclear what direct role autophagy plays in IBD pathogenesis.[39, 40]

The gut mucosa is an important physical, chemical, and immunological interface between the gut microbiota and the host; thus any dysregulation or breakdown in this barrier could contribute to disease.[41, 42] Altered physical epithelial

barrier function, thinner mucus layer, and altered responses to endoplasmic reticulum (ER) stress (via mutations in *MUC19, ITLN1, FUT2,* and *XBP1*) have all been identified as risk-associated loci for IBD.[43–46]

The observation that many of the identified genetic risk loci in IBD implicate genes involved in the complex interplay between a host and gut microbes points to a central role for host-microbe interactions at the root of IBD pathogenesis.

THE HUMAN GUT MICROBIOME

The human gut microbiota consists of a complex consortium of microorganisms that include members of the superkingdom of Archaea, Bacteria, and Eukarya.[47] It remains possible that any member or consortia of the microbiota might be associated with or even cause IBD. To date, much of the work has been focused on the bacterial community within the gut microbiota due in large part to the evolution of technology using next-generation sequencing to study the composition and function of the bacterial population within the gut microbiota. Focusing on the bacteria will help us understand the technological and bioinformatic challenges that will need to be applied to the study of other members of the gut microbiota, including fungi and viruses.

The composition of the gut microbiota and its functional relationship with the host is an important determinant of health and disease. With the advent of the Human Microbiota Project (HMP), defining the composition and function of the microbiota in healthy subjects, we are beginning to understand the nature of this ecological niche as it relates to the human host.[48, 49] The determinants of the composition of the microbiota include environmental factors such as exposure to pollution, diet, and exposure to drugs, especially antimicrobials.[50–53] Most importantly, the inoculum delivered to the newborn infant during childbirth seems to dictate the microbiota composition during the critical period of development in early life, during which the immune system and gut microbes develop a bidirectional relationship.[54–57] A stable gut microbial composition is achieved between 1 and 3 years of age, and it remains stable unless there is a major perturbation such as an illness, use of antibiotics, or major changes in diet.[54, 58–60] Although the environment is a major influence on the microbiota, it is also apparent that the gut microbial composition is influenced by host genetic factors.[51, 61, 62]

Heritability of the Microbiota

The gut microbiome seems to be influenced by hereditable factors, as is evident from twin studies and studies of large cohorts of healthy subjects. Monozygotic twins have a more similar microbiome than dizygotic twins, with dizygotic twins being more similar than unrelated individuals.[59, 63–65] Several studies have now shown that elements of the microbiome, including specific taxa, are heritable. The largest heritability study on the gut microbiome was performed in a cohort of 416 mostly female twin pairs from the United Kingdom, with a mean age of 60 years, thus reducing the potential confounding effect of a shared environment on the microbiome.[66] This study identified 26 taxa to be heritable (using a nominal $P < 0.05$), with *Christensenella* being the taxa with the highest heritability score. These 26 heritable taxa were mostly replicated, and new heritable taxa were identified in 2 separate large cohorts.[67, 68] In a third study of 271 related healthy individuals from 123 pedigrees, we showed that almost a third of the bacterial taxa in stool were heritable.[51] The finding that there are familial influences on the bacterial composition raises the possibility that specific host genetic variants may account for the individual variability in microbial profiles.

Association of IBD Risk–Associated SNPs and Gut Microbiota in Healthy Subjects

To assess possible genetic associations with microbiota composition, several approaches have been taken. One study focused on the IBD risk–associated SNPs and their associations with microbiome composition in 582 healthy individuals.[69] It found that a high genetic risk for IBD was associated with a decrease in the relative abundance of the acetate-to-butyrate converter *Roseburia*.[69, 70]

To our knowledge, no study has been able to identify a robust association of *NOD2* genetic polymorphism with microbial taxa in healthy human subjects. Nonetheless, a suggestive association ($4.6 \times 10^{-6} < P < 1.3 \times 10^{-4}$) was identified in a cohort of 1514 healthy individuals.[62] In that study, the *NOD2* locus was associated with the enterobactin biosynthesis pathway, which is highly correlated with *Escherichia coli* abundance. The authors suggested that this pathway could help *E. coli* to evade the host innate immune responses in inflammatory gut disease.[62, 71]

Among the genes involved in barrier function, the protein encoded by the alpha[1, 2]-fucosyltransferase 2 gene (*FUT2*), which is responsible for secretion of the ABO histo-blood group antigens in the mucosa, may be involved in alterations of the gut microbiota. The minor allele (A) confers a nonsecretor phenotype that is associated with CD susceptibility, with an odds ratio of 1.1 for CD (95% CI, 1.071–1.143; $P < 10^{-15}$).[4] Early studies have shown that *FUT2* polymorphisms were associated with the microbiome.[72–74] Similar results were confirmed more recently in a cohort of 33 healthy individuals[75]; however, a larger study of 1503 healthy twins[76] could not replicate these findings. Therefore, replication studies with larger cohorts are necessary to define the effects of any associations between host genotype and microbial composition.

GWAS of Microbiota In Healthy Individuals

In an effort to define the genetic basis of the hereditability of the gut microbiome, GWAS between a host's genetics and their microbiome composition have been performed. The first such GWAS used the Human Microbiome Project metagenomics data.[77] Here, the high sequencing depth of fecal DNA

allowed for identification of host genetic polymorphisms.[52] Despite the small sample size (93 individuals), they identified several associations between host genetics and microbial taxa. The most convincing was the association of rs56064699 located in the *LCT* gene, which encodes the lactase gene responsible for lactose hydrolysis. The minor allele of rs56064699 was associated with an increase in the relative abundance of *Bifidobacteria*.[52] This association was later replicated in a small cohort of healthy Hutterites.[78] It is possible that these initial studies have identified associations with higher effect size than are likely to be seen in subsequent studies, due in part to the phenomenon referred to as the winners-curse effect.[79]

The gut microbiome can also be assessed for diversity, such as alpha diversity, which measures the presence/absence of unique taxa within a sample, whereas beta diversity assesses the presence and abundance of taxa between samples.[80–83] Alpha diversity of the microbiome is decreased in both UC and CD patients compared with healthy controls.[80–83] As measured by the Shannon index, this parameter was shown to be heritable; however, to our knowledge, no SNPs have been associated with microbial alpha diversity,[51, 78] suggesting that environmental exposures might have a larger effect on this parameter compared with host genetics.

Another proxy for an "IBD-like microbiota" is the microbial dysbiosis index (MD-index) described by Gevers et al.[84] This index is defined based on measures of altered microbial composition in the ileum and was suggested to be specific to the diagnosis of CD.[84] Even though this index showed a moderate score for heritability, a GWAS in healthy subjects failed to identify any significant association between SNPs and the MD-index.[51] Several SNPs did show a nominal association with the MD-index, for example, rs2138126, rs2138125, and rs2589132 ($P < 1.4 \times 10^{-6}$), all located within the regulatory associated protein of *MTOR* complex 1 (*RPTOR*), which is involved in immunity and obesity.[85, 86] In addition, among all of the suggestive associations (ie, nominal associations with $0.05 > P > 10^{-8}$), rs11575056 ($P = 1.9 \times 10^{-6}$) were also associated with the MD-index.[4] This is potentially interesting because rs11575056 is located close to the chemokine (C-C motif) ligand 8 (*CCL8*), an important inflammatory response chemokine,[87] and next to a known IBD risk region (17q12).

Another study used 2 population-based cohorts, 1 from Schleswig-Holstein (Germany) comprised of the 914 healthy individuals recruited from the PopGen study and a second with 1115 individuals recruited from the FoCus study, comprised of 371 obese subjects from the FoCus obesity cohort, to identify SNPs associated with microbial composition.[61] Their data suggested that host genetics contributes to overall bacterial composition,[61] with 42 loci associated with microbiome composition, as measured by the Bray-Curtis beta diversity index. Here rs7974353, which is located within the vitamin D receptor (*Vdr*) locus, accounted for a small fraction (0.75%) of the microbiota variation in the combined cohort. Carriers of homozygote TT of the rs7974353 *Vdr* variant had a decrease in the relative abundance of *Parabacteroides*.[61] Vitamin D receptor has been associated with an increased risk of IBD,[88–90] particularly the homozygote minor genotype for the *Vdr* (TaqI polymorphism at codon 352 of exon 8), which is more frequent in CD patients compared with UC patients or healthy controls.[88] Altogether, these results suggest that genetic variants of *Vdr* modulate the *Parabacteroides* relative abundance and that this interaction might influence IBD risk.[91] It is of note that they also showed an upregulation of VDR expression in biopsies of CD and UC patients with acute inflammation as compared with healthy controls. This upregulation was accompanied by a lower relative abundance of *Parabacteroides*, possibly supporting such an interaction.[61]

In a study by Bonder et al., 42 SNPs were associated with bacterial function and composition using metagenomic shotgun sequencing of 1514 healthy subjects.[62] In this study, 9 loci were associated with specific bacterial taxa and 33 were associated with bacterial function using a conventional *P* value threshold for GWAS ($P < 5 \times 10^{-8}$). Other associations were related to IBD loci, but with nonsignificant *P* values ($<5 \times 10^{-4}$). Among these, the rs2155219 located in the C11orf30–LRRC32 locus is part of a cell-cell signaling pathway (gene ontology [GO] term: 0007267) and was previously associated with IBD risk with an OR of 1.15.[4] Interestingly, using the same data set, this GO term was correlated with the abundance of 2 potentially IBD-associated bacteria, *Coprococcus comes* and *Proteobacteria*.[62] Other IBD-associated genes were identified as associated with the gut microbiome function: *CCL2* associated with phosphopantothenate biosynthesis III, *DAP* associated with sucrose degradation IV (sucrose phosphorylase), and *IL23R* associated with 4-chlorobenzoate degradation.[62]

In a large discovery cohort comprised of healthy FDRs of CD patients, we identified 58 SNPs that were associated with 33 bacterial taxa, and 4 of these associations were replicated in a second mixed cohort of European ancestry comprised of Canadian, American, and Israeli.[51] When IBD risk–associated SNPs were analyzed in this cohort, no statistical association was observed after correction for multiple testing.[51] This cohort is enriched in IBD risk–associated variants as the cohort was composed of healthy FDRs of CD patients; therefore, one would expect that the power to detect associations of IBD risk–associated SNPs would be greater than in the general healthy population.[14] Given that none of the recent GWAS papers identified IBD risk loci associated with bacterial taxa, it is tempting to conclude that genetic influence on the microbiome composition is independent of IBD genetic risk. Interestingly, our study identified that the relative abundance of *Faecalibacterium,* a taxa frequently decreased in IBD as compared with healthy controls, is influenced by rs1394174 located within the *CNTN6* gene. This suggests that the relative abundance of *Faecalibacterium* may be driven both by host genetics and environment factors.[51, 67] Moreover, the association

of rs1394174 with *Faecalibacterium* was replicated in an independent cohort of 696 healthy individuals.[92]

The analysis of host genetic influence on the gut microbiota is still in its infancy, and the findings described highlight the complexity of this analysis and the potential pitfalls of working with relatively small cohorts. Important covariates have frequently not been included in GWAS analysis of the microbiota. For example, it is known that stool frequency and consistency are associated with microbiota composition.[93, 94] Many other factors such as diet, antibiotic use, and disease diagnosis also influence microbiota.[95–97] Further, it should be highlighted that the presence of inflammation in patients with established disease will further confound the apparent associations. Larger-scale analysis or meta-analysis is required to fully define the effect of host genetics on the gut microbiome. Toward this end, the MiBioGen consortium will combine data from multiple centers currently comprising more than ~19,000 individuals (Wang et al., unpublished data). Whether these SNP-microbiome associations identified in healthy subjects can be implicated in the triggering of IBD onset remains to be shown. The finding that there are associations between host genetic makeup and gut microbiota leads us to speculate that the gut microbiota may serve as a heritable vector influencing the onset of IBD.

Associations of Host Genetics and the Gut Microbiome in IBD Patients

Few studies have investigated the effect of host genetics on the gut microbiome in individuals diagnosed with IBD. This is probably because inflammation itself is a major confounder and strongly influences the composition of the microbiota.[1] Thus, it is much more challenging to identify any specific effect of host genetics (especially if it has a small effect size) on the microbiota of individuals with an inflamed gut. Despite this fact, in 474 IBD patients, there was an association between *NOD2* risk allele dosage, comprised of rs104895431, rs104895467, rs2066844, rs2066845, rs5743277, rs5743293, and the microbiota from intestinal biopsies, particularly with *Enterobacteriaceae*.[98] As this association is not observed in healthy subjects, this finding suggests that *NOD2* polymorphisms may be important in defining host-microbiome interactions along the gastrointestinal tract during inflammation.[51, 69]

In a small study of 9 CD patients homozygous for the T300A risk allele in *ATG16L1*, the patients had impaired clearance of potential pathobionts, including *Bacteroidaceae*, *Enterobacteriaceae,* and *Fusobacteriaceae*.[99] This implies that the *ATG16L1* polymorphism associated with CD could alter the microbiota composition through alterations in secretion of antibacterial peptides[39, 40]; however, in a study of 313 patients with IBD, the IBD-GRS (based on 200 IBD risk–associated SNPs) showed no association with microbial composition.[69] These results suggest that host genetics can influence the microbiome composition but that inflammation can mask or alter this association.

Microbiome Alterations in IBD

Given the observation that fecal diversions in CD patients can decrease the inflammatory process and that inflammation in mouse models of colitis is dependent on the presence of a gut microbiota, it has long been proposed that the gut microbiome plays a significant role in IBD. Identifying alterations in the gut microbiota of IBD patients could provide insight into the influence of the gut microbiome on the etiology of IBD.[100] In an attempt to define the microbiota in patients with IBD, most studies have compared IBD cases with healthy controls (Fig. 1, Table 1). Studies attempting to identify a single bacterium or a group of bacteria as causal have failed. Nonetheless, the attempts to identify specific changes in the microbial composition in IBD patients using case-control studies have led to a number of interesting findings. IBD patients tend to have reduced abundance of bacteria belonging to the phyla of Firmicutes and Bacteroidetes, while being enriched for bacteria from the phyla of Proteobacteria and Actinobacteria.[101, 102] A decreased relative abundance of *Bacteroides*, *Faecalibacterium*, *Roseburia*, *Blautia, Ruminococcus*, and *Coprococcus*, in addition to other taxa within the families of *Ruminococcaceae* and *Lachnospiraceae,* was reported in several studies in CD.[82, 84, 103] On the other hand, the family of *Enterobacteriaceae* is increased in IBD.[82, 84, 104] Two members of this family, *Enterococcus* and *Escheriachia coli,* are increased in CD[105, 106] and UC.[107] Other changes that are often reported include a decrease in the genera *Bifidobacterium*,[45, 108] *Prevotella*,[109] and *Coprococcus*,[102, 106] as compared with healthy controls. How these described alterations relate to the underlying nature of IBD, either CD or UC, remains to be determined. In order to conclude whether any of these changes occurs in IBD patients independent of the inflammatory process, or before the development of intestinal inflammation, will require a prospective cohort study of high-risk individuals before disease onset.

Nevertheless, the association between adherent-invasive *Escherichia coli* (AIEC) and the development of CD in the terminal ileum is worth discussing. AIEC is a member of the *Enterobacteriaceae* family, and this family has been reported to be increased in the majority of the studies assessing microbial variations in CD patients compared with healthy controls.[131, 132] On the other hand, a decrease in the relative abundance of *Faecalibacterium prausnitzii* has also been described in CD and UC.[102, 121, 133] This taxon, along with *Roseburia hominis* (another "protective" taxon decreased in CD patients), can produce butyrate, which has anti-inflammatory properties.[83, 121, 123, 126, 134]

Studies that have looked more broadly at changes in the absolute abundance of mucosa-associated bacteria (as assessed by the total number of 16S rDNA gene copies) have shown an increase in subjects with IBD as compared with non-IBD, suggesting that IBD patients may have increased bacterial

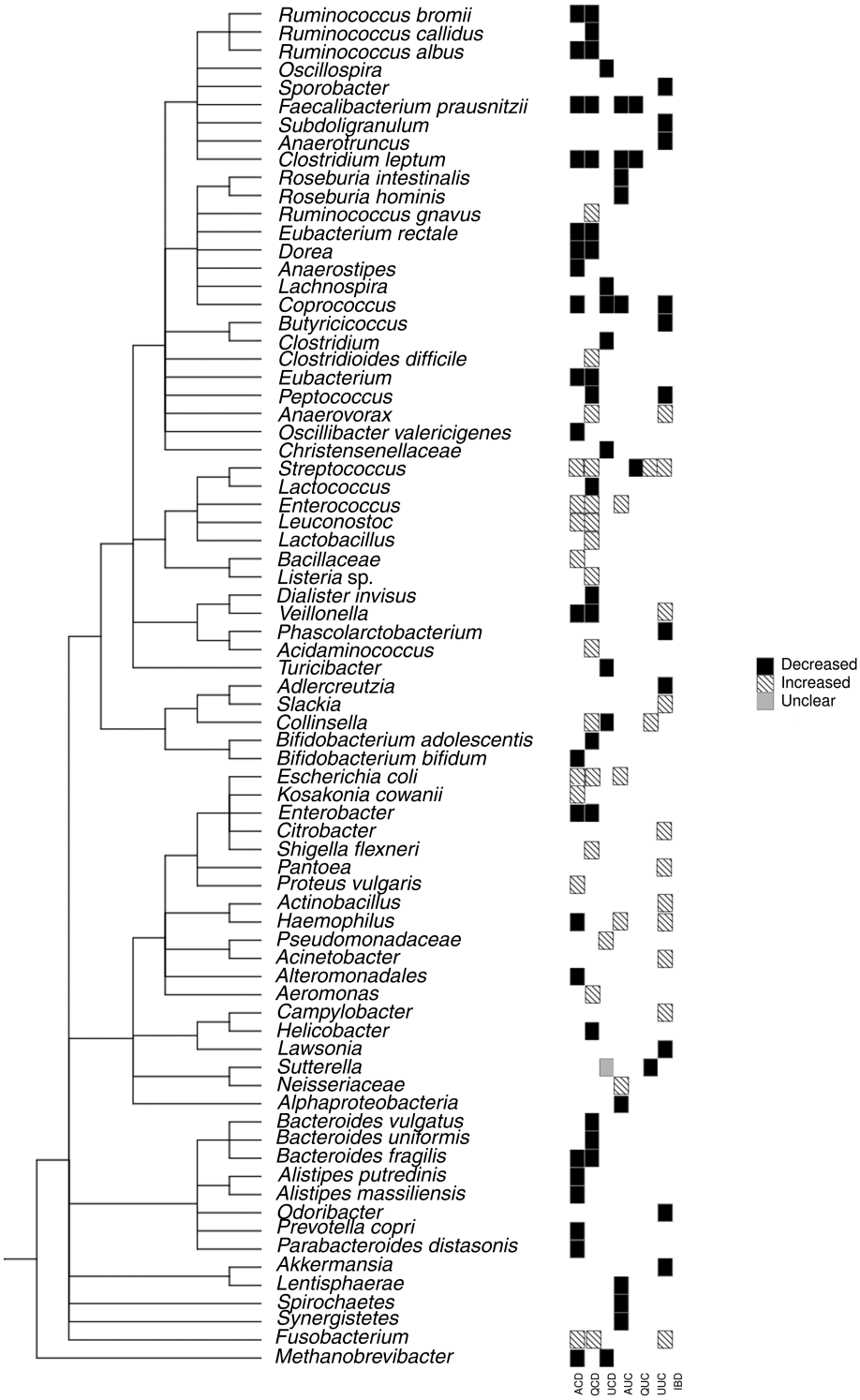

FIGURE 1. This phylogenetic tree presents a summary of the described variation of a selection of taxa from publications assessing the fecal microbiome from active CD (ACD), quiescent CD (QCD), unspecified CD (UCD), active UC (AUC), quiescent UC (QUC), unspecified UC (UCD), and IBD patients. In the heat map, black case indicates the taxa that are described as significantly decreased in most (>50%) of the assessed studies in diseased patients, striped cases signifies that the taxa are significantly increased, and the grey means that variations are contradicting between the assessed studies (50% of studies have contradictory results). The plot was generated as follows: Taxa names were first collected from publications and searched for on the NCBI Taxonomy database. The Taxonomy IDs were then imported to the PhyloT web interface (http://phylot.biobyte.de/) to generate a phylogenetic tree (for better visibility, the internal nodes were collapsed). The final plot was then generated in R using the ggtree and the gheatmap commands from the ggtree package.

TABLE 1: Fecal Bacterial Taxa Associated With IBD

Taxa	Increase	Decreased	Similar	Disease Activity
Total diversity		103, 105, 106, 108–115	109C	Active CD
		80, 105, 108, 110, 112, 114, 116	109	Quiescent CD
		117		Unspecified CD
		103, 106, 111, 112, 118	113	Active UC
		112	114	Quiescent UC
Aeromonas	82I			Quiescent CD
Acidaminococcaceae		106		Active CD
Acidaminococcus	82I			Quiescent CD
Acinetobacter	103			IBD
Actinobacteria		118		Active UC
Actinobacillus	103			IBD
Adlercreutzia		103		IBD
Akkermensia		115		IBD
Alistipes		115		IBD
Alistipes putredinis		119		Active CD
Alistipes massiliensis		109		Active CD
Alphaproteobacteria		118		Active UC
Alteromonadales [Chromatiaceae]		109		Active CD
Anaerosptipes		114		Unspecified CD
Anaerovorax		115		IBD
	82I			Quiescent CD
Anaerotruncus		115		IBD
Bacillaceae	106			Active CD
Bacteroidaceae		117		Unspecified CD
	106			Active UC
Bacteroides	108	105	111	Active CD
	108		80, 108	Quiescent CD
		106	111	Active UC
Bacteroides fragilis		110		Active CD
		110, 120		Quiescent CD
Bacteroides uniformis		116a		Quiescent CD
Bacteroides vulgatus		120		Quiescent CD
Bacteroidetes		119		Active CD
		118		Active UC
Betaproteobacteria		118		Active UC
Bifidobacteria		105	110, 111	Active CD
	82		110	Quiescent CD
			111	Active UC
Bifidobacterium		108	121	Active CD
		108		Quiescent CD
			121	Active UC
Bifidobacterium adolescentis		122		Quiescent CD
Bifidobacterium bifidum		119		Active CD
Blautia	106	84		Active CD
	114			Unspecified CD
Butyricicoccus		115		IBD
Camplylobacter	103			IBD

(Continued)

TABLE 1: Continued

Taxa	Increase	Decreased	Similar	Disease Activity
Citrobacter	103			IBD
Clostridiaceae	114			Unspecified CD
Clostridiales		109R		Active CD
		114		Unspecified CD
Clostridium		114		Unspecified CD
Clostridium coccoides group		80		Quiescent CD
		111, 123		Active UC
			123	Quiescent UC
Clostridium cluster XIVa		108, 117		Active CD
		108, 122		Quiescent CD
Clostridium difficile	120			Quiescent CD
Clostridium leptum subgroup		110–112, 119, 121		Active CD
		80, 110, 112, 121		Quiescent CD
		112, 121, 123		Active UC
		112	121	Quiescent UC
Collinsella	82			Quiescent CD
		114		Unspecified CD
	114			Unspecified UC
Coprococcus		106, 109		Active CD
		106		Active UC
		114		Unspecified CD
		103		IBD
Coriobacteriaceae		84		Active CD
Christensenellaceae		114		
Deltaproteobacteria		118		Active UC
Dialister	114			Unspecified CD
Dialister invisus		122		Quiescent CD
Dorea		84, 117		Active CD
		117		Quiescent CD
Enterobacter cowanii	119			Active CD
Enterobacteriaceae	82I			Quiescent CD
	117			Unspecified CD
	106			Active UC
	103, 115			IBD
Escherichia	117, 124	108		Active CD
	117	108		Quiescent CD
	114			Unspecified CD
	106	113		Active UC
	103, 115			IBD
Escherichia coli	119, 120		119	Active CD
	120			Quiescent CD
	121			Active UC
Enterobacter		108		Active CD
		108		Quiescent CD
Epsilonproteobacteria	118			Active UC
Erysipelotrichaceae	117	114		Unspecified CD
	103			IBD
Eubacteriaceae	106			Active CD

TABLE 1: Continued

Taxa	Increase	Decreased	Similar	Disease Activity
Eubacterium		108		Active CD
		108		Quiescent CD
Eubacterium rectale		119		Active CD
		120		Quiescent CD
Enterobacteria	105		111	Active CD
	105			Quiescent CD
			111	Active UC
Enterococcus	106			Active CD
	120			Quiescent CD
	106			Active UC
Enterococcaceae	106			Active CD
	106			Active UC
	103			IBD
Faecalibacterium		108, 109, 117, 124R, 106		Active CD
	82C	82I		Quiescent CD
		114		Unspecified CD
Faecalibacterium prausnitzii		112, 121, 125		Active CD
		112, 119, 120, 122, 125		Quiescent CD
		112, 121, 123, 126		Active UC
		112, 127	123	Quiescent UC
Firmicutes		80		Quiescent CD
		118		Active UC
Fusobacteria	106, 117			Active CD
		118		Active UC
Fusobacterium	117			Active CD
	82I, 114	82C		Quiescent CD
	103			IBD
Gammaproteobacteria	117			Active CD
	117			Unspecified CD
	118			Active UC
Haemophilus		113		Active CD
	113			Active UC
	103			IBD
Helicobacter	128			Quiescent CD
Lachnospiraceae		109R, 117		Active CD
		117		Unspecified CD
Lachnospira		114		Unspecified CD
Lactobacillaceae	106			Active CD
Lactobacillales	84			Active CD
	114			Unspecified CD
	103			IBD
Lactobacillus			121	Active CD
	82I			Quiescent CD
			121	Active UC
Lactococcus		82C		Quiescent CD
Lawsonia		115		IBD
Lentisphaerae		118		Active UC
Listeria sp.	120			Quiescent CD

(Continued)

TABLE 1: Continued

Taxa	Increase	Decreased	Similar	Disease Activity
Leuconostoc	[108]			Active CD
	[108]			Quiescent CD
Methanobrevibacter		[109]		Active CD
		[114]		Unspecified CD
Mogibacteriaceae		[114]		Unspecified CD
Neisseriaceae	[106]			Active UC
Odoribacter		[115]		IBD
Oscillibacter		[115]		IBD
Oscillibacter valericigenes		[119]		Active CD
Oscillospira		[114]		Unspecified CD
Pantoea	[103]			IBD
Pasteurellaceae	[106]			Active UC
Parabacteroides		[115]		IBD
		[114]		Unspecified CD
Parabacteroides distasonis		[119]		Active CD
Peptococcus		[117]		IBD
		[82]I		Quiescent CD
Peptostreptococcaceae		[114]		Unspecified CD
Phascolarctobacterium		[115]		IBD
Prevotellaceae		[117]		Unspecified CD
Prevotella	[108]	[117]		Active CD
		[117]	[108]	Quiescent CD
		[82]		Quiescent UC
Prevotella copri		[109]		Active CD
Proteus	[108]			Active CD
	[108]			Quiescent CD
Proteus vulgaris	[119]			Active CD
Proteobacteria	[106]			Active CD
	[106, 118]			Active UC
	[117]			Unspecified CD
Pseudomonadaceae	[117]			Unspecified CD
Rikenellaceae		[115]		IBD
		[106]		Active CD
	[114]			Unspecified CD
Roseburia		[106, 117]		Active CD
		[82, 117]		Quiescent CD
		[114]		Unspecified CD
Roseburia hominis		[126]		Active UC
Ruminococcaceae		[106, 109]		Active CD
		[114, 117]		Unspecified CD
Ruminococcus		[108, 109R, [84, 106]		Active CD
	[82]	[108]		Quiescent CD
	[114]	[114]		Unspecified CD
		[103, 115]		IBD
Ruminococcus albus		[119]		Active CD
		[120]		Quiescent CD
Ruminococcus bromii		[119]		Active CD
		[120]		Quiescent CD

TABLE 1: Continued

Taxa	Increase	Decreased	Similar	Disease Activity
Ruminococcus callidus		[120]		Quiescent CD
Ruminococcus gnavus	[122]			Quiescent CD
Ruminococcus intestinalis		[123]		Active UC
			[123]	Quiescent UC
Shigella	[117, 124]			Active CD
	[106, 117]			Quiescent CD
	[115]			Active UC
Shigella flexneri	[120]			Quiescent CD
Slackia	[103]			IBD
Spirochaetes		[118]		Active UC
Sporobacater		[115]		IBD
Streptococcaceae	[84, 106]			Active CD
	[117]			Unspecified CD
Streptococcus	[84, 106, 108, 117]			Active CD
	[108, 117]			Quiescent CD
		[82]		Quiescent UC
	[114]			Unspecified UC
	[103]			IBD
Sutterella	[114]	[117]		Unspecified CD
		[114]		Unspecified UC
Subdoligranulum		[115]		IBD
Synergistetes		[106]		Active UC
Turicibacter		[114]		Unspecified CD
Verrucomicrobiale		[115]		IBD
		[118]		Active UC
Veillonellaceae		[117]		Unspecified CD
Veillonella		[108, 117]		Active CD
	[82I]	[82C, 108]		Quiescent CD
	[103]			IBD

Abbreviations: I, ileal CD; C, colonic CD; R, resection.
[a]Significant for ileal CD only. Microbiota was analyzed using different methods including dot blot hybridization,[105] DGGE,[110] fluorescence in situ hybridization (FISH) adapted to flow cytometry,[111] T-RFLP,[108] quantitative polymerase chain reaction (qPCR) and TTGE,[112] cloning and full-length 16S rRNA gene sequencing,[113] V4 sequencing of the bacterial 16S rRNA,[103, 109, 114] Sanger sequencing,[106, 115] 454 pyrosequencing,[82, 106, 116] macroarray,[80] terminal restriction fragment length polymorphism (T-RFLP)[80, 108, 116] microarray,[118] qPCR and microarray,[119] microarray,[120] qPCR,[121, 125, 127, 128] DGGE,[122] V4 sequencing of the bacterial 16S rRNA and shotgun sequencing,[84] FISH,[123] 16S sequencing,[124] qPCR and DGGE.[126] Articles were selected based the reviews published by Li et al.[129] and Ni et al.[130]

translocation into the gut mucosa.[104, 135] On a larger scale, studies of the composition of the gut microbiota in IBD patients have suggested that there is a generalized imbalance in microbiome composition as compared with healthy controls, sometimes referred to as "dysbiosis" in IBD. Again, It is difficult to know if dysbiosis is a cause or a result of the disease process, as the inflammatory process itself can alter the composition of the microbiota.[1] Aerobic microbes in IBD are increased as compared with nondisease conditions,[45, 136] perhaps due to the increase in the reactive oxygen species generated during inflammation that react with endogenous luminal sulphur compounds to form new respiratory electron acceptors. This can, for example, favor the growth of species resistant to reactive oxygen species similar to *Salmonella typhimurium*.[137] This same phenomenon could be implicated in the alteration of the other aerobic microbes in the gut of IBD patients.

ENVIRONMENTAL DETERMINANTS OF RISK FOR IBD

There may be other factors influencing IBD that seem to have a hereditable influence. Environmental factors and demographic factors, such as smoking and exposure to second-hand smoke, urban vs rural life,[138] air pollution,[139] and cultural influences on diet,[140] all represent shared exposures that may constitute familial risk for IBD.[141] Many of these factors may explain the apparent association between "Westernisation" and the risk

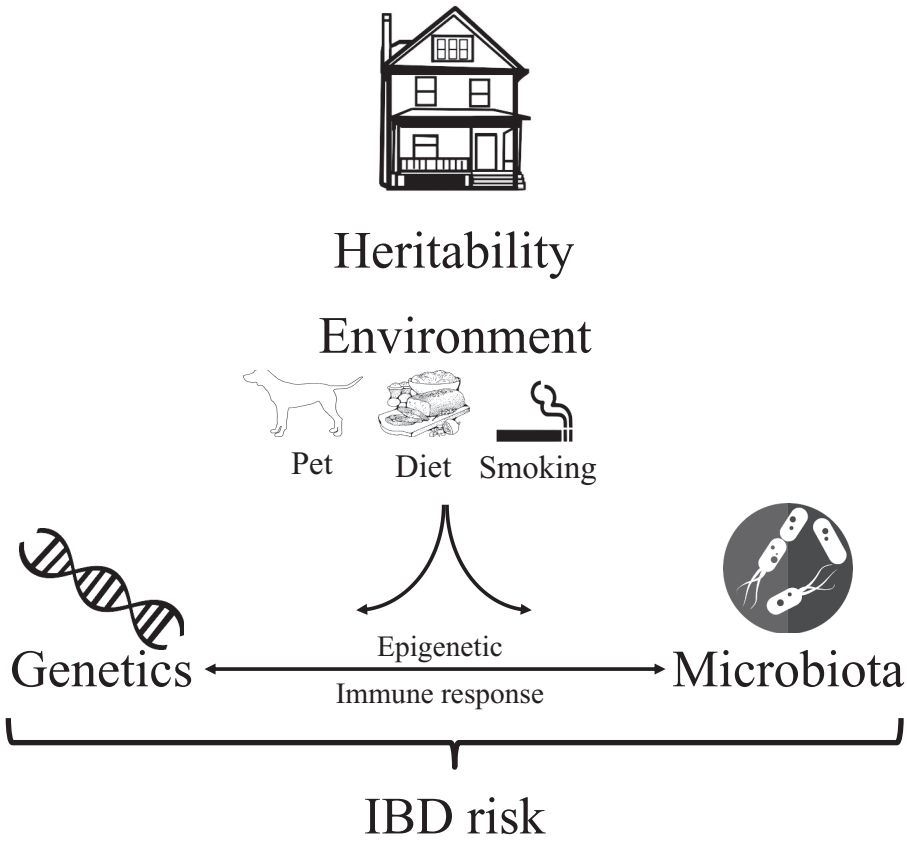

FIGURE 2. Factors that combine to explain the heritability of IBD. In addition to host genetics, the microbiome might contribute to heritability of IBD. Nongenetic components that are shared in families of relatives of IBD patients include dietary and cultural habits; pet exposure can also influence the IBD risk through influencing the microbiota.

of IBD development, as has been described in China and offspring of South Asian immigrants to North America.[142] These observations again raise the issue of how cultural influences, such as diet, play a role in modulating the risk of IBD in certain ethnic communities and in certain geographic locations. It will be important to identify the role of these environmental influences and factors in IBD.[143]

Smoking has been associated with IBD risk, specifically with the risk of CD,[144, 145] and is associated with increased intestinal permeability,[146] but what remains unclear is whether the impact is mediated through the gut microbiome.[147] It also remains unclear whether second-hand smoke exposure can increase risk of IBD onset. A meta-analysis failed to identify a relationship between childhood passive smoke exposure and CD[148]; however, more recent studies have contradictory results, thus the influence of second-hand smoke on IBD onset requires further investigation.[149–151]

Diet is a major factor capable of modifying gut microbiota composition.[95, 152] Indeed, as part of the IBD European Prospective Cohort Study (IBD-EPIC study), many dietary factors have been associated with IBD onset.[153–156] Milk consumption was shown to be potentially associated with a decreased risk of developing CD (P = 0.23 for CD) but not with UC (P = 0.60),[154] whereas a role of flavones and resveratrol in the risk of developing CD was also observed.[155] The IBD-EPIC study findings also support a role for dietary linoleic acid in the etiology of UC.[155] In this cohort, obesity, as measured by body mass index (BMI), was not associated with the development of UC or CD.[156] Overall, total fiber intake from fruit, vegetables, or cereals, and the subsequent development of either CD or UC were not associated.[153] However, it is important to acknowledge that most of these analyses are based on food frequency questionnaire data that may have important limitations.[158] As meals and diet are usually shared between parents and their children from the same household, the influence of diet on the gut microbiota profile can seem heritable.

Epigenetics

The term "epigenetics" refers to heritable changes in phenotype that occur independently of changes to the DNA sequence. Epigenetic modifications include DNA methylation and histone modifications.[159] The concept that IBD can be influenced by epigenetically induced changes in gene expression is supported by the fact that GWAS have identified many

IBD-associated variants adjacent to genes encoding DNA methyltransferases and their interacting proteins.[4] For example, the mutation or differential expression of the *uhrf1* gene has been linked to DNA methylation changes in mammals and zebrafish.[160] As a result of inactivation or expression modulation of this gene in zebrafish, the DNA methylation changes lead to an IBD-like phenotype.[161–164]

Epigenetic modifications are known to be influenced by environmental factors such as diet and the microbiota,[165] particularly in colorectal cancer.[166] Indeed, the gut microbiota was shown to alter host histone acetylation and methylation in human colon tissues[167] and in gene promoters linked to inflammatory responses such as *IL23* or *IgA*.[168] Moreover, fermentation end products, especially short-chain fatty acids such as acetate, butyrate, and propionate, produced by microbial fermentation of fiber, may be important for the epigenetic regulation of inflammatory reactions.[167]

Epigenetics is likely to play a role in IBD pathogenesis; however, the changes in inflammatory diseases remain to be defined.[159] There are several challenges in studying epigenetics in IBD. Epigenetic signatures are cell type specific[165]; however, a standardized database of methylated genes in IBD have been recently released[169] and will help research into the epigenetic basis of disease. Determining whether an IBD epigenetic signature exists would require that future studies consider several crucial aspects such as isolation of disease-relevant cell types obtained from carefully selected cohorts of patients with homogenous disease location and activity.[165] Currently, most of the studies on epigenetics in IBD focus on UC patients and are mainly focused on methylation of DNA from biopsies or DNA derived from peripheral blood.[170, 171] Studies on CD patients are only starting to emerge.

Unlike GWAS studies, which require large cohorts to detect genetic variations, previous studies on DNA methylation in IBD patients were able to detect several methylated positions using much smaller cohorts.[165] This might indicate that epigenetics may provide much stronger signals as compared with GWAS. If epigenetics proves to be important in IBD pathogenesis, future studies on focused subsets or phenotypes of IBD patients might get around the issue of disease heterogeneity. Nonetheless, early studies using only 18 patients with CD compared with 25 healthy controls identified as many as 4287 differentially methylated positions in DNA derived from peripheral blood cells, indicating that CD patients display a specific methylation "landscape."[172] Another study found that 3196 probes were differentially methylated in DNA derived from peripheral blood mononuclear cells (PBMCs) from 149 IBD cases and 39 controls. After enrichment analysis, the authors showed that the differentially methylated probes were significantly associated with 104 GO terms. Interestingly, in CD, the most enriched pathways identified involve immune responses, regulation of T-cell activation, and cellular response to molecules of bacterial origin,[173] suggesting that epigenetic modification is involved in microbial and immune response dysregulation in IBD. Another study has compared intestinal epithelial cells purified from 66 IBD patients to 30 non-IBD controls and showed that there were distinct and stable patterns of DNA methylation in the 2 groups.[171] An alternative approach to study epigenetic mechanisms is the development of ex vivo intestinal organoid–based analysis, which has already shown promising results despite limited sample size.[171]

Altogether, multidimensional analysis of the complex immune cell infiltrates in intestinal tissue of IBD patients harbors the key to understanding disease pathogenesis and how this contributes to the perceived hereditability of these diseases.[174] Despite technical difficulties in obtaining homogenous cohorts and biopsy samples, epigenetics has the potential to provide an explanation to the missing heritability of IBD.

CONCLUSION

What can be defined as heritability of IBD can be explained by the fact that host genetics and the microbiome contribute to IBD. It is reasonable to suggest that the missing heritability, that is, the occurrence of IBD in families not explained by genetic factors, could be explained by the interaction of host genetics with the microbiota under certain environmental conditions. GWAS have identified several genetic variants linked to IBD that are shared across different ethnic groups, and that the list of risk-associated SNPs will probably keep increasing. In addition to defined genetic risk, the microbiota, which can be influenced by host genetics and environmental factors, plays a role. Thus, genetic risk and microbiome composition certainly contribute to the heritability of IBD. In addition, nongenetic components such as shared environmental factors in individuals from the same household, for example, smoking or pet exposure, could contribute to the onset of IBD (Fig. 2). Overall, the search for a "cure" for IBD requires appreciating the complex interplay between host genetics, gut microbes, environmental triggers, and the mucosal immune response.

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