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Genetics and pathogenesis of inflammatory bowel disease

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Abstract

Recent advances have provided substantial insight into the maintenance of mucosal immunity and the pathogenesis of inflammatory bowel disease. Cellular programs responsible for intestinal homeostasis use diverse intracellular and intercellular networks to promote immune tolerance, inflammation or epithelial restitution. Complex interfaces integrate local host and microbial signals to activate appropriate effector programs selectively and even drive plasticity between these programs. In addition, genetic studies and mouse models have emphasized the role of genetic predispositions and how they affect interactions with microbial and environmental factors, leading to pro-colitogenic perturbations of the host–commensal relationship.

Inflammatory bowel disease (IBD) comprises the chronic relapsing inflammatory disorders Crohn's disease and ulcerative colitis. Family history is a risk factor for developing IBD, with a peak incidence in early adult life, although individuals of any age can be affected. IBD is thought to result from an inappropriate and continuing inflammatory response to commensal microbes in a genetically susceptible host. Recent progress in understanding IBD pathobiology offers insight into relevant disease mechanisms in mucosal immunity, including how genetic factors interact with microbial and environmental cues within tissue-specific contexts, the biological checkpoints involved, the selective decisions made during the course of disease and how plasticity of the biological response results in the capacity for different phenotypes.

Ulcerative colitis is characterized by inflammation that is limited to the colon: it begins in the rectum, spreads proximally in a continuous fashion and frequently involves the periappendiceal region. By contrast, Crohn's disease involves any part of the gastrointestinal tract — most commonly the terminal ileum or the perianal region — in a non-continuous fashion and, unlike ulcerative colitis, is commonly associated with complications such as strictures, abscesses and fistulas. Histologically, ulcerative colitis shows superficial

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inflammatory changes limited to the mucosa and submucosa with cryptitis and crypt abscesses. The microscopic features of Crohn's disease include thickened submucosa, transmural inflammation, fissuring ulceration and non-caseating granulomas.

Among complex diseases, genome-wide association studies (GWAS) have been successful in IBD, identifying 99 non-overlapping genetic risk loci, including 28 that are shared between Crohn's disease and ulcerative colitis[1,2] (Fig. 1). The genes implicated in childhood-onset and adult-onset IBD overlap, suggesting similar contributory genetic predispositions and pathophysiological pathways. Adding to the complexity of understanding disease mechanisms, a susceptibility allele often requires other genetic and non-genetic cues to manifest disease. The concordance rate in monozygotic twins of 10–15% in ulcerative colitis compared with 30–35% in Crohn's disease suggests that non-genetic factors may have an even more important role in ulcerative colitis than in Crohn's disease[3]. Furthermore, the higher penetrance of common Crohn's-disease-associated polymorphisms in genetic case-control studies than in population-based studies of cohorts of the same ethnicity is probably due to the concomitant aggregation of both genetic and environmental factors in the case-control studies[4]. Smoking is an example of a disease-specific modifier that seems to exacerbate Crohn's disease while being protective against ulcerative colitis. Evidence suggests that smoking impairs autophagy, a process thought to be involved especially in Crohn's disease, demonstrating how exposure to a disease modifier in a genetically predisposed individual may mechanistically affect IBD development[5].

In this Review, we provide an overview of genes and susceptibility loci implicated in IBD by GWAS and other genetic studies. Candidate genes are discussed in the context of IBD-relevant pathways, as well as how these molecular pathways interact with environmental factors to modulate intestinal homeostasis.

Genes and pathways in IBD

International collaborative research groups focusing on an unbiased appraisal of the human genome have been particularly successful in identifying genes and genetic loci that contribute to IBD susceptibility[1,2]. Despite distinct clinical features, approximately 30% of IBD-related genetic loci are shared between ulcerative colitis and Crohn's disease, indicating that these diseases engage common pathways and may be part of a mechanistic continuum (Fig. 1).

Analyses of the genes and genetic loci implicated in IBD show several pathways that are crucial for intestinal homeostasis, including barrier function, epithelial restitution, microbial defence, innate immune regulation, reactive oxygen species (ROS) generation, autophagy, regulation of adaptive immunity, endoplasmic reticulum (ER) stress and metabolic pathways associated with cellular homeostasis (Fig. 2). Early studies have suggested the existence of both protective and predisposing alleles[6]. Disease-relevant biological pathways are further highlighted when several components are implicated as risk factors together (Fig. 3).

Multidisease comparative analysis can uncover common disease-causing genes and pathways. More than 50% of IBD susceptibility loci have also been associated with other inflammatory and autoimmune diseases. These overlapping genes can have contrasting effects in different diseases. For example, the same coding variant of *PTPN22* (R620W) is a strong risk factor for type 1 diabetes and rheumatoid arthritis, but is protective against Crohn's disease[7]. These data suggest that crucial clues to disease biology may reside in understanding the function of these shared genes. Several loci containing genes such as *MST1*, *IL2*, *CARD9* and *REL* are shared between ulcerative colitis and the associated complication primary sclerosing cholangitis (PSC)[8]. This overlap may help to identify subsets of patients with ulcerative colitis who are at risk of PSC. Risk loci for Crohn's

disease present an unexpected overlap with susceptibility regions for *Mycobacterium leprae* infection, including genes such as *NOD2*, *C13orf31* and *LRRK2* (ref. 9). Although absent from the leprosy GWAS, other Crohn's-disease-associated genes are also implicated in host responses to mycobacterial infection, including *CARD9*, *LTA*, *ITLN1* and *IRGM*[1]. Thus, studies to delineate immune responses to antigens from, and infection by, mycobacteria, or other microbes that elicit similar host cell responses, may also be pertinent to Crohn's disease.

Genetic variants associated with IBD can vary in frequency depending on the cohort ethnicity, raising the possibility that some such variants may have emerged in the context of historical selective pressures. Although this notion remains to be demonstrated in IBD, lessons from other autoimmune and infectious contexts lend support. For example, variants of apolipoprotein L1 and the inhibitory Fc receptor FcγRIIb that confer protection against trypanosomiasis and malaria, respectively, are more common in populations endemically exposed to these pathogens, but these variants also confer increased susceptibility to focal segmental glomerulosclerosis and systemic lupus erythematosus (SLE), respectively[10,11].

Current GWAS are typically powered to characterize variants of >1% frequency and do not include the contributions from rare variants (<1% frequency). Exome sequencing can be useful for identifying rare variants, whereas whole-genome sequencing is of value in elucidating modifier loci. If pedigrees are available, rare variant discovery can be further targeted by fine mapping, as shown by the identification of *IL10RA* polymorphisms associated with the development of early-onset IBD[12]. Other interleukin-10 receptor (IL-10R) signalling components have also been implicated by GWAS, including *STAT3*, *TYK2*, *JAK2* and *IL10* itself, in concordance with the notion that both rare and common variants may highlight the same pathway. Although these components can also function in other contexts — for example, the transcription factor STAT3 and the kinase proteins TYK2 and JAK2 are involved in the signalling of the interleukins IL-6, IL-22 and IL-23 — these results illustrate the value of genetic studies in determining not just single genes, but also disease-relevant pathways. Recent resequencing studies in IBD recovered both known and new variants of *CARD9*, *NOD2* and *IL23R*, with independent effects on disease risk. The *IL23R* variants were protective, supporting previous findings of a common protective *IL23R* allele and illustrating how studies of rare variants can reinforce GWAS findings[6]. Furthermore, T helper 17 (T_H17) cells generated *ex vivo* from subjects with a variant *IL23R* allele (R381Q) show decreased production of the pro-inflammatory cytokine IL-17A in response to IL-23 stimulation, emphasizing the importance of IL-23-related pathways in human IBD[13].

Early functional studies attempting to determine causality have largely focused on coding variants, although non-coding single nucleotide polymorphisms (SNPs) can be associated with qualitative and quantitative changes. Alternative splicing exemplifies a qualitative change affected by non-coding modifications. In the context of regulating immune responses, *IL23R* and *NOD2* can encode truncated variants that inhibit their signalling pathways[14,15]. Furthermore, genetic changes may affect transcription-factor-binding sequences, locus accessibility, translational efficiency and *trans*-regulators such as non-coding RNAs and microRNAs (miRNAs). In this regard, a Crohn's-disease-associated synonymous variant in *IRGM* (c.313C>T) perturbs regulation by miR-196A and miR-196B, and is associated with altered IRGM expression in patients with Crohn's disease who bear this SNP[16]. *Cis*- or *trans*-expression quantitative trait loci (eQTL) are detected for approximately half of the IBD risk regions, indicating that allele-specific gene-expression changes contribute to disease risk (Fig. 1). Furthermore, IBD-implicated loci contain more than 10 miRNA-encoding sequences and 39 large intervening non-coding RNAs (lincRNAs), 5 of which interacted with the histone methyltransferase polycomb repressive

complex 2 (PRC2), supporting the notion that regulation of gene expression by miRNAs and lincRNAs may be mechanistically relevant in IBD[17].

So far, GWAS account for 23% and 16% of the heritability in Crohn's disease and ulcerative colitis, respectively[1,2]. Although these may be underestimates owing to the net effect of common variants that are individually too small to calculate accurately; the missing heritability may further comprise genetic, epigenetic and non-genetic (including environmental) components. Genetic factors such as rare variants, private mutations, structural variants and interactions between genes are not well captured by GWAS. Nevertheless, a key success of GWAS in IBD has been the ability to provide insight into disease pathobiology by highlighting key molecular pathways.

Epithelial encounters and pathogenicity

The intestinal mucosa exists in a functional equilibrium with the complex luminal milieu, which is dominated by a spectrum of microbial species and their products. Maintaining this functional balance is central to preserving normal mucosal physiology, with perturbations contributing to the pathophysiology of many gastrointestinal disorders, including IBD. In addition to nutrient absorption, intestinal epithelial cells (IECs) perform both barrier and signal-transduction functions, with the capacity to sense luminal contents through surface receptors and, in return, secrete regulatory products that can orchestrate an appropriate response in the underlying lamina propria.

Molecular details of the epithelial barrier and the structure of tight junctions, which are crucial to its integrity, have been characterized. Abnormal intestinal permeability has been observed in IBD patients and in some of their first-degree relatives. Genes within several IBD-associated loci indicate a role for barrier integrity in disease predisposition, implicating candidate genes such as *CDH1*, *GNA12* and *PTPN2*. Genetic studies have shown that truncated forms of the adherens junction protein E-cadherin (encoded by *CDH1*) are associated with Crohn's disease, and intestinal biopsies from patients with Crohn's disease carrying these mutant alleles show inappropriate protein localization and cytosolic accumulation[18]. Activation of the G protein $G\alpha_{12}$ (encoded by *GNA12*) leads to phosphorylation of the tight junction proteins ZO-1 and ZO-2, resulting in destabilization of cell junctions in epithelial cell lines[19]. *In vitro* studies show that the protein tyrosine phosphatase family member PTPN2 protects against interferon-γ (IFN-γ)-induced epithelial permeability; concordantly, *Ptpn2*-deficient mice show increased susceptibility to experimental colitis[20,21].

Genetic studies have associated IBD with several transcription factors involved in epithelial regeneration, such as HNF4A and NKX2-3, which control crypt cell proliferation and IEC differentiation, respectively[22–24]. Spontaneous colitis did not occur in all animal models with IEC-specific deletion of *Hnf4a*, suggesting that further environmental triggers are required for disease[22,23]. STAT3, the gene encoding which lies within an IBD-implicated locus, is activated in epithelial cells from patients with IBD, and IEC-specific *Stat3* deletion affects epithelial repair[25].

The intestinal barrier is enhanced by the presence of a pre-epithelial layer formed primarily of mucus glycoproteins, trefoil peptides, IgA and antimicrobial peptides (AMPs). Goblet cells generate the mucus layer, a protective polysaccharide bilayer rich in cationic proteins, the inner layer of which is essentially devoid of microbes. Patients with IBD frequently have a compromised mucus layer and increased mucolytic bacteria; mucus layer defects are also observed in $Muc2^{-/-}$ and IEC-specific $C1galt1^{-/-}$ mice, which develop spontaneous colitis[26]. Interestingly, some patients with ulcerative colitis show defective intestinal O-glycosylation resembling that seen in $C1galt1^{-/-}$ mice[26]. Paneth cells are located in the

crypts of the small intestine. In addition to the role of these cells in crypt homeostasis and maintenance of the intestinal stem-cell niche, they also secrete antimicrobial effectors that prevent microbial invasion and control the composition of the gut microflora. These effectors include lysozyme, RegIIIγ, secreted phospholipase A_2 (which degrades bacterial membrane phospholipids) and defensins HD5 and HD6 (pore-forming hydrophobic peptides that can integrate into bacterial membranes, resulting in lysis). Production of AMPs is regulated by Toll-like receptor (TLR) and NOD2 signals triggered by commensal flora. Paneth cell defects and susceptibility to intestinal inflammation have been uncovered in mice deficient in several Crohn's-disease-associated genes, including *Nod2*, *Atg16l1* and *Xbp1* (refs 27–29). These results highlight pathways important to Paneth cell biology, such as the regulation of AMP production (*Nod2*), granule exocytosis (*Atg16l1*) and the ER stress response (*Xbp1*). Similar phenotypes have been observed in human disease, such that patients with Crohn's disease carrying the *ATG16L1* (T300A) mutation show Paneth cell granule abnormalities. These findings suggest that defects in Paneth cell biology may define a subset of patients with Crohn's disease.

Cells with high synthetic capacity and secretory activity, such as Paneth cells and goblet cells, have high baseline levels of ER stress, leading to activation of the unfolded protein response (UPR), which controls cellular programs that allow proper protein processing. The UPR is mainly cytoprotective, although it can signal apoptosis after sustained ER stress. Increased intestine epithelial ER stress and susceptibility to colitis have been observed in mice with overactivation of, or perturbations in, the UPR pathway, including *Muc2* missense mutation, *Agr2*$^{-/-}$, *Ern2*$^{-/-}$ (also known as *Ire1b*$^{-/-}$), IEC-specific *Xbp1*$^{-/-}$ and *Mbtps1*-hypomorphic mice[29,30]. Similarly, studies in primary IECs from patients with IBD show activated ER stress responses, and hypomorphic variants of XBP1 have been associated with risk of IBD[29]. Overall, these results indicate that genetic variants that perturb mechanisms that protect against ER stress can affect intestinal homeostasis in IBD. In addition to its effects on cell viability, ER stress also activates autophagy and IL-23 release, suggesting that sustained ER stress may engage inflammatory circuits that are subsequently propagated by T cells[31].

In addition to limiting bacterial translocation across the mucosal barrier, IECs promote intestinal homeostasis by regulating innate and adaptive immune responses. Illustrating this point, IECs produce intestinal alkaline phosphatase, which can mediate lipopolysaccharide detoxification. Resolvin-E1, which is generated in part through the action of epithelial cyclooxygenase-2, attenuates neutrophil transmigration and upregulates epithelial expression of intestinal alkaline phosphatase during the restitutive response, a process termed epithelial imprinting[32]. IECs can also modulate adaptive immune responses, driving the differentiation of anti-inflammatory T regulatory (T_{reg}) cells by releasing the vitamin A metabolite retinoic acid and the cytokines thymic stromal lymphopoietin (TSLP) and transforming growth factor-β (TGF-β)[33]. Breakdown in such epithelial defence mechanisms could lead to pathological intestinal inflammation.

Checkpoints in the innate immune response

The physical barrier of the intestinal epithelium is complemented by a well-evolved mucosal innate immune system, which is populated by cells poised to defend against pathogenic incursions and curtail inflammatory responses to maintain a state of hyporesponsiveness to commensal bacteria. Dendritic cells, macrophages, innate lymphoid cells (ILCs) and neutrophils are crucial cellular components of the innate immune system during infection or inflammation. Supporting the notion that defective innate immune responses can lead to IBD, patients with innate immunodeficiencies such as chronic granulomatous disease and Hermansky–Pudlak syndrome, which is associated with defective responses to bacterial

DNA motifs (CpG oligonucleotides) specifically in plasmacytoid dendritic cells, tend to develop IBD[34]. Similarly, patients with Crohn's disease have defective innate immune responses, including attenuated macrophage activity *in vitro*, and impaired neutrophil recruitment and exogenous *Escherichia coli* clearance *in vivo*[35].

Intestinal dendritic cells constitute a central interface for monitoring the environment and relaying signals to initiate appropriate adaptive immune responses[33]. Dendritic cell subsets are specialized and respond to endogenous and exogenous stimuli such as microbial motifs, fatty acids, oxidized lipids and vitamin D by selectively engaging pro-inflammatory, anti-inflammatory, epithelial restitutive or T-cell education programs, as well as inducing IgA production[33,36]. For example, T_{reg}-cell differentiation can be promoted by tolerogenic dendritic cells induced by TSLP, TGF-β and retinoic acid, all of which are made by IECs and stromal cells; these dendritic cells express the integrin CD103 but not the chemokine receptor CX_3CR_1 (ref. 33). By contrast, dendritic cells expressing E-cadherin are a pro-inflammatory and page 298 subset that promotes T_H17-cell differentiation (see ref. 37 for further details). Bacterial flagellins can override dendritic cell tolerogenic programs by stimulating TLR5 and inducing the release of pro-inflammatory mediators from hyporesponsive lamina propria $CD11c^{high}$ dendritic cells, pointing to a broader role for flagellated bacteria in IBD[38]. This specific immunostimulatory role for TLR5 may be particularly relevant in IBD, as seroreactivity to the bacterial flagellin CBir1, observed in approximately 50% of patients with Crohn's disease, correlates with a complicated clinical course.

Intestinal homeostasis is maintained in part by the actions of resident macrophages that have enhanced phagocytic and bactericidal activity and decreased production of pro-inflammatory cytokines. Specialized macrophage subsets are also involved; tumour-necrosis factor-α (TNF-α)-secreting and IL-1β-secreting $Ly6C^{high}$ monocytes are recruited in the initial phase of microbial challenge or tissue injury, whereas reparative IL-10-secreting, TGF-β-secreting and vascular-endothelial-growth-factor-secreting $Ly6C^{low}$ monocytes are mobilized during the resolution phase of inflammation[39]. Neutrophils may also contribute to the resolution of inflammation, for example, by synthesizing anti-inflammatory mediators such as lipoxin A_4. Studies showing impaired secretion of lipoxin A_4 in mucosal tissues from patients with ulcerative colitis support the relevance of such mechanisms in IBD.

IL-22 is emerging as an important cytokine in epithelial homeostasis, showing protective activity in different models of colitis through its stimulatory effect on antimicrobial and reparative processes. Produced by several cell types, such as ILCs, lymphoid tissue induced (LTi) cells, T_H17 cells and γδ T cells, most intestinal IL-22 at steady state is produced by ILCs expressing the transcription factor RORγt[40,41]. Studies in patients with Crohn's disease have shown decreased frequencies of IL-22-secreting ILCs in the lamina propria[42]. Together, these findings suggest a central role for ILCs (and other IL-22-producing cells) in regulating intestinal homeostasis, which remains to be characterized in IBD.

NOD2 and IBD

NOD2 was the first gene to be associated with IBD, and thereafter several genes that interact epistatically with *NOD2* signalling were also implicated. NOD2 recognizes the peptidoglycan product muramyl dipeptide (MDP), which modulates both innate and adaptive immune responses[43] (Fig. 4). For example, MDP stimulation induces autophagy, which controls bacterial replication and antigen presentation, and acts on dendritic cells in conjunction with TLR ligands to promote T_H17-cell differentiation[44,45]. NOD2 may also contribute to immune tolerance. These effects are impaired in cells from patients with the Crohn's-disease-associated *NOD2* mutation 3020insC. Furthermore, NOD2 can participate in distinct MDP-independent pathways such as regulation of the T-cell response and the type

I IFN response to single-stranded RNA (ssRNA) stimulation, indicating that gut microbial ssRNAs may exist and have immunomodulatory properties[46]. The relative contributions of these cytosolic MDP-sensing pathways vary greatly between cell types (Fig. 4). Further studies are needed to uncover the effect of disease-associated *NOD2* alleles in different cell-specific programs, and unravel the precise role(s) of NOD2 in IBD. Other families of innate immune receptors linked to intestinal inflammation and immunity include NOD-like receptors (NLRs) and RIG-I-like receptors (RLRs). These receptors recognize microbial motifs or damage-associated molecular patterns and can activate the inflammasome, thus appropriate regulation of these pathways is required for intestinal homeostasis. For example, mouse knockout studies of *Nlrp3* or *RIG-I* (also known as *Ddx58*) show increased susceptibility to experimental colitis[47]. Conversely, sustained overactivation of NLRs can also have detrimental effects, as illustrated by activating mutations in NOD2 and NLRP3 giving rise to Blau syndrome and cryopyrinopathies, respectively.

CARD9 and IBD

CARD9 is an IBD-implicated adaptor protein that integrates signals from many innate immune receptors that recognize viral, bacterial and fungal motifs. Depending on the stimulus, CARD9 interacts with distinct signalling complexes and activates different pathways to modulate cytokine environments appropriately[48,49]. In particular, recognition of fungal motifs in human dendritic cells leading to CARD9 and dectin-1 signalling results in the broad activation of members of the nuclear factor-κB (NF-κB) transcription factor family, whereas CARD9 and dectin-2 signalling selectively activates the IBD-implicated NF-κB factor REL, enhancing the production of T_H17-polarizing cytokines such as IL-1β and the IL-23 p19 subunit[50]. Defective CARD9 function leads to the immune disorder mucocutaneous candidiasis, at least in part owing to failure to promote an adequate T_H17 immune response. These data illustrate how innate immune signalling molecules, including NOD2 and CARD9, can act as central hubs to integrate diverse signals and selectively activate specific effector pathways; in the polymicrobial context of the gut, it seems reasonable that defects at such nodal points would constitute key predispositions to IBD.

Redox equilibrium in IBD

The reduction and oxidation (redox) state of the gut depends on an equilibrium between oxidants, such as free radicals, ROS or reactive nitrogen species, and antioxidant mechanisms, such as the glutathione peroxidase (GPX) and glutathione *S*-transferase enzymes. This redox state affects many signal-transduction pathways, such as NF-κB signalling and AMP activity[51]. Supporting the importance of antioxidant pathways in intestinal homeostasis, mice deficient in both *Gpx1* and *Gpx2* develop spontaneous colitis. IBD genetic studies have implicated loci containing *GPX1* and *GPX4*, further highlighting the relevance of these mechanisms in disease (Fig. 2). Among the oxidants, ROS represent an important class of effector molecules generated by mitochondrial and non-mitochondrial sources. ROS are non-toxic at basal levels and are even required to maintain the intestinal stem-cell niche. In the context of innate immunity, ROS have important antimicrobial activity, and contribute to intracellular signalling, promoting the production of pro-inflammatory cytokines. Furthermore, ROS generated by epithelial cells after infection can transmit signals to adjacent cells in a paracrine manner, allowing the local coordination of chemokine production[52]. Genes within several IBD-associated loci may either regulate ROS production or protect against oxidative stress (Fig. 2). In particular, NOD2, CARD9 and IFN-γ-regulated leucine-rich repeat kinase 2 (LRRK2) all contribute to ROS production[43,53,54]. In addition to pro-inflammatory pathways, ROS are also involved in T_{reg}-cell polarization and function[55,56]. Thus, understanding the role of disease variants will require a broader understanding of the cell- and tissue-specific effects of ROS.

Autophagy and IBD

Genetic analyses have shown an unsuspected role for autophagy in innate immunity and IBD, implicating two component genes, *ATG16L1* and *IRGM*, in IBD pathogenesis[57–59]. Autophagy is involved in intracellular homeostasis, contributing to the degradation and recycling of cytosolic contents and organelles, as well as to resistance against infection and the removal of intracellular microbes (Fig. 5). ATG16L1 is essential for all forms of autophagy, and the coding mutation T300A is associated with increased risk of Crohn's disease. Despite ubiquitous expression of *ATG16L1*, defects associated with *ATG16L1* polymorphisms have so far been described only within the gut, probably owing to the high microbial load in this tissue. Subsequent evidence for MDP stimulation of NOD2-activated autophagy illustrates a link between genetic risk loci, and highlights the importance of defining disease-associated pathways and the potential of new roles for known genes[44,45]. Epithelial cells and dendritic cells containing Crohn's-disease-associated *ATG16L1* and *NOD2* variants show defects in antibacterial autophagy[44,45,60]. In dendritic cells, these defects are associated with an impaired ability to present exogenous antigens to $CD4^+$ T cells[44]. These results illustrate a close relationship between NOD2, ATG16L1 and autophagy, affecting intracellular processing and communication with the adaptive immune system, suggesting that genetic polymorphisms may affect both pathways concomitantly.

Abnormalities consistent with Crohn's disease have been observed in mice with defects in autophagy, including hypomorphic *Atg16l1* (*Atg16l1*^{HM}) and IEC-specific *Atg5*-deficient mice[27]. Paneth cells either from *Atg16l1*^{HM} mice or from patients with Crohn's disease who have the *ATG16L1* (T300A variant) allele show aberrant granule size, number and location, and reduced AMP secretion; notably, they also show gain of function, as evidenced by upregulated peroxisome proliferator-activated receptor signalling[27]. The landmark findings that gnotobiotic (germ-free) *Atg16l1*^{HM} mice lost these Paneth cell anomalies and their sensitivity to dextrate sulphate sodium (DSS)-induced colitis, and that these abnormalities were restored by norovirus infection provide a definitive demonstration of how host–microbial interactions contribute to the pathophysiology of IBD[61].

Effectors and regulators of adaptive immunity

Homeostasis in the gut involves a balance between anti-inflammatory and pro-inflammatory signals, such that inflammatory disease results from an inadequate T_{reg}-cell response in the face of an overly exuberant response largely involving T_H1 and T_H17 cells in Crohn's disease and T_H2 cells in ulcerative colitis. Intestinal inflammation resulting from a failure to maintain this balance is exemplified by patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome or with *WAS* (also known as *WASP*) deficiency, who have deficient T_{reg}-cell function. Furthermore, T_H-cell polarization in IBD is unlikely to be a simple divergence between a few disparate T-cell fates, but rather to include diverse sub-programs that can be selectively activated by antigen-presenting cells, cytokine milieu, microbial factors and metabolic programs. This notion is supported by the findings that T cells expressing both IFN-γ and IL-17 are detected during all stages of Crohn's disease, and it evolves our understanding of pro-inflammatory and anti-inflammatory cell types and pathways.

Recent studies indicate that T_{reg} cells and T_H17 cells may arise from a common precursor, consistent with the observation that TGF-β helps to direct differentiation of both subsets[62]. The generation of two subsets with opposing activities from a common precursor is reminiscent of differential responses of precursor cells along morphogen gradients during development, suggesting that similar morphogen gradients may work in the gut *in vivo*. In this regard, TGF-β alone drives T_{reg}-cell differentiation, with retinoic acid exerting a synergistic effect; the differentiation and function of mucosal T_{reg} cells depends on the

transcription factors BLIMP1 and IRF4 (ref. 63). Given the abundance of TGF-β in intestinal tissues, this may contribute to baseline homeostasis, for example, by promoting T_{reg}-cell differentiation in naive lamina propria $CD4^+$ T cells. However, in conjunction with other signals, including cytokines, metabolites and microbial signals, T_H17-cell differentiation is promoted instead. Experiments demonstrating the crucial role of IL-23, IL-6 and IL-17 in the development of experimental colitis support a role for T_H17 cells in disease propagation[64]. Illustrating some of the intercellular interactions that affect the T_H17–T_{reg}-cell axis, γδ T cells can drive the T_H17 program and contribute to experimental colitis, and are in turn suppressed by T_{reg} cells[65,66]. Furthermore, T_{reg} cells can support the development of T_H17 cells by maintaining decreased levels of IL-2 in the local milieu[67].

Transcriptional programs helmed by the T_{reg}- and T_H17-cell-lineage-defining transcription factors FOXP3 and RORγt work together with a network of transcription factors, which can in turn respond to lineage-inducing cytokines and microbial factors. Transcription factors can mediate dichotomous functions depending on the cellular (and probably cytokine) context; for example, STAT3 drives T_H17-cell differentiation, but is also required for anti-inflammatory IL-10 signalling through distinct pathways, inducing repressors such as strawberry notch homologue 2 (SBNO2). The aryl hydrocarbon receptor (AHR) is a nuclear receptor that is essential for IL-22 production and also enhances IL-17 production, albeit to a lesser extent than RORγt-driven pathways, illustrating how distinct transcription factors may drive separate functions in the same cell[68]. AHR responds to polycyclic hydrocarbons, suggesting that xenobiotic stimuli may modulate IL-22 and IL-17 production. Many of the genes required for T_{reg}- and T_H17-cell differentiation have been implicated in IBD (Fig. 3). *CCR6*, which lies in a locus associated with Crohn's disease, encodes a chemokine receptor that is important for lymphocyte homing to the gut as well as for the development of intestinal lymphoid follicles — areas important for the production of T-cell-independent IgA, which affects the microbiota composition of the host[33]. Thus, the gut may use TGF-β pathways to poise T cells to carry out both pro- and anti-inflammatory programs depending on the local presence of cytokines and microbial products.

Illustrating the concept that hyper- or hypo-activation can affect the outcome of T-cell differentiation, defects in ITCH, a HECT-type E3 ubiquitin ligase involved in T-cell activation, lead to impaired T_{reg}-cell polarization in mice and to autoimmunity in patients[69]. IBD-associated loci also contain other members of the ITCH pathway, including NDFIP1 and TNFAIP3. Defects in these proteins are associated with inappropriate T-cell activation, skewed T_H-cell polarization and pathological intestinal inflammation, consistent with the hypothesis that *NDFIP1* and *TNFAIP3* are disease-contributory genes[70].

The soluble mediators secreted by T_{reg} cells are also released by other, $FOXP3^-$ regulatory T-cell subsets in the gut, such as T regulatory 1 (T_r1) cells. IL-10 release can be induced by IL-27 in several subsets, including both pro-inflammatory T_H1 and anti-inflammatory T_r1 subsets. IL-27 is made by antigen-presenting cells, illustrating another homeostatic interaction between innate and adaptive immune cells[71]. Interestingly, GWAS implicate both *IL10* and *IL27* in IBD, suggesting that this may represent a central axis of immune regulation in the context of IBD.

In addition to the contribution of T-cell subsets, experimental evidence suggests that B-cell defects may contribute to the development of colitis in several ways, including impaired IgA production and antigen presentation, effects on early B-cell selection, and the perturbed production of pro- and anti-inflammatory mediators. Supporting the importance of IgA production in IBD, recent GWAS of selective IgA deficiency showed genes also implicated in IBD, namely *ORMDL3*, *REL* and *PTPN22* (ref. 72).

Immunoglobulins can also have immune modulatory activity, which is highlighted by IBD genetic studies. Peripheral B-cell tolerance can be maintained by signalling through the lectin CD22, which binds immunoglobulin bearing α2,6-linked sialic acid[73]. This epitope is generated in part through the action of sialic acid acetylesterase (SIAE). Sequencing studies in small cohorts detected rare SIAE variants that disrupted enzyme function and secretion in patients with IBD and other autoimmune diseases[73]. In addition, sialylated IgG may signal through DC-SIGN (dendritic-cell-specific ICAM3-grabbing non-integrin) on myeloid cells, leading to increased expression of inhibitory FcγRIIβ (the gene encoding which lies in an ulcerative-colitis-implicated locus) on macrophages. These data demonstrate pathways by which immunoglobulins can exert anti-inflammatory activities and highlight components that genetic studies suggest may be perturbed in IBD.

The functional relevance of anti-inflammatory B regulatory (B_{reg}) cells has been demonstrated in several mouse models of inflammatory diseases, including colitis; B_{reg} cells from patients with SLE also show impaired function[74]. B_{reg} cells differentiate after stimulation in the context of either anti-CD40 antibody or TLR ligands, and secrete anti-inflammatory cytokines such as TGF-β and IL-10. Defects in B_{reg}-cell development or function might lead to failure to upregulate IL-10, leading to attenuated suppression of $CD4^+$ T-cell production of IFN-γ and TNF-α, consistent with a broader role of B_{reg} cells in autoimmunity and inflammatory disease.

Other cell types that help to regulate gut immunity include intraepithelial lymphocytes, which comprise many subsets such as $CD8\alpha\alpha^+$ γδ T cells (which show both cytoprotective and cytolytic activities) and $CD8\alpha\alpha^+$ αβ T cells (which are thought to be regulatory cells that require TGF-β for development)[75]. Enrichment analysis of expression profiles further suggests that a subset of IBD-implicated genes is expressed in natural killer T (NKT) cells, which can detect infection though microbial lipids presented by CD1d or through atypical endogenous lipids, such as isoglobotriaosylceramide, which accumulate after microbial–TLR signalling[76]. Furthermore, NKT cells from patients with ulcerative colitis produce IL-13 and show enhanced cytotoxicity, further indicating that a perturbed NKT-cell response to as yet unidentified bacterial ligands may be pro-colitogenic.

Genetic studies may offer insight into how this balance is disturbed, leading to pathological inflammation. Interestingly, perturbation by blocking cytotoxic T-lymphocyte antigen 4, an important inhibitory molecule expressed on activated T cells, commonly resulted in colitis in patients, again suggesting the poised state of activation of T cells in the gut[77].

Mucosal ecology and immune responses in disease

The gut microflora is a community that has co-evolved with the host and confers beneficial effects, including helping to metabolize nutrients, modulate immune responses and defend against pathogens. However, dysregulation of normal co-evolved homeostatic relationships between gut bacteria and host immune responses can lead to intestinal inflammation. Indeed, accumulating evidence suggests that luminal flora is a requisite, perhaps even a central factor in the development of IBD.

Efforts to correlate changes in enteric microbial communities with disease are complicated by the great interindividual variation, such that even monozygotic twins may share only 40% of faecal phylotypes[78]. However, clustering the abundance of genes in certain categories in a species-independent fashion shows high interindividual similarity, suggesting that the microbiome can be perceived as a conserved functional entity[79]. Differences in the abundance of both bacterial species and functional gene categories (such as bacterial motility, sugar and iron metabolism) can differentiate patients with IBD from healthy

individuals, demonstrating IBD-related changes in gut microbial ecology[80] (C. Huttenhower, personal communication).

Even within an individual, intestinal microbial communities are dynamic and influenced by host factors, dietary effects and the microbes themselves. Many of the specific examples have emerged from studies in mice. Illustrating how microbial communities can be affected by host responses, infection-induced inflammation results in an oxidative metabolic shift in *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium), which the bacterium uses in conjunction with host-derived ROS to create a growth advantage over fermenting bacteria[81]. Microbe–host relationships are tightly interrelated, such that host factors can induce functional changes in the microflora that, in return, affect host biology. TLRs recognize microbial motifs and have a crucial role in determining mucosal susceptibility to injury and repair responses. Impaired TLR signalling due to *Myd88* deficiency in non-obese diabetic (NOD) mice induces changes in microbiota community structure that protect against diabetes[82]. Conversely, impaired innate immune function in *T-bet*$^{-/-}$*Rag1*$^{-/-}$ (also known as *Tbx21*$^{-/-}$*Rag1*$^{-/-}$) mice and *Tlr5*$^{-/-}$ mice leads to the generation of a pathogenic microbiota that causes colitis and metabolic syndrome, respectively, even in genetically normal hosts[83,84]. Similarly, the gut microbiota induces a dynamic IgA response, with qualitative and/or quantitative defects in IgA production resulting in impaired control of the microbial communities[85,86]. Deficiencies in activation-induced cytidine deaminase — an enzyme essential for somatic hypermutation and class-switching recombination during B-cell maturation — result in IgA deficiency, specific expansion of the anaerobic flora and segmented filamentous bacteria (SFB), and overstimulation of the mucosal immune system, with hyperplasia of mucosal lymphoid structures such as Peyer's patches and lymphoid follicles. Similar observations in mice with selectively impaired somatic hypermutation point to the importance of affinity maturation in generating diversity in the IgA repertoire to control the intestinal microbial burden[86].

Mice fostered on milk lacking sialyl(α2,3)lactose develop a distinct microbiota that confers transmissible resistance to DSS colitis, providing an example of dietary effects on the gut microbiota[87]. Dietary glycans can also be incorporated onto host cell membranes and can act as receptors for bacterial toxins. These findings demonstrate that host factors, both transient and genetic, can act together with dietary factors to modulate microbiota community structure and/or function, sometimes indelibly, in IBD-relevant ways.

The intestinal mucosa can monitor microbial ligands using pattern recognition receptors (PRRs), and microbial metabolites using G-protein-coupled receptors (GPCRs) and solute carriers. Short-chain fatty acids (SCFAs), generated by some microflora constituents and decreased in ulcerative colitis, can signal through the receptor GPR43 in neutrophils, with notable proresolving effects on inflammation[88]. Other examples of GPCRs with immune modulatory activity include GPR120, GPR65 (also known as TDAG8) and GPR35, which can be activated by ω-3 fatty acids, extracellular protons and kynurenic acid, respectively, with anti-inflammatory effects[89]. Of interest, kynurenic acid and other anti-inflammatory kynurenines are generated by the catabolism of tryptophan. Host levels of tryptophan are affected by the microbiota, suggesting how microbes can modulate the host immune response by metabolic effects. Other microbial metabolites can be transported into host cells. For example, the solute carrier SLC22A5 transports a quorum-sensing molecule from *Bacillus subtilis*, conferring resistance to oxidative stress *in vitro*[90]. The proton-coupled histidine/peptide cotransporter SLC15A4 is required for TLR7 and TLR9 signalling in plasmacytoid dendritic cells[34]. Furthermore, studies in *Slc15a4*$^{-/-}$ dendritic cells suggest that SLC15A4 contributes to TLR9 signalling by regulating endosomal histidine levels, and to NOD1 signalling by cytosolic delivery of NOD1 ligands such as the tripeptide motif L-Ala-γ-D-Glu-meso-diaminopimelic acid (Tri_{DAP}). *Slc15a4* deficiency ameliorates

susceptibility to DSS colitis in mice[91]. These findings and the presence of genes such as *SLC22A5*, *GPR35* and *GPR65* in IBD-risk loci suggest that PRRs, GPCRs and solute carriers help to maintain microbe–host relationships and intestinal homeostasis by transducing signals from microbial ligands and metabolites, which can in turn have immune modulatory effects on the host.

Microbial signals also shape innate and adaptive immune responses. Germ-free animals have underdeveloped Peyer's patches, as well as fewer IgA-producing plasma cells and lamina propria $CD4^+$ cells, illustrating the role of the microbiota in generating a mature mucosal adaptive immune response. The constituents of the microbiota can have important protective roles; for example, the impaired epithelial injury response in *Myd88*-deficient mice highlights the role of microbial stimulation in epithelial restitution[92]. Similarly, in mice, commensal bacteria activate expression of the transcription factor NFIL3, inhibit *Il12b* expression and protect against colitis, and $CD14^+$ lamina propria cells from patients with IBD express less NFIL3 than healthy controls[93]. The microbiota also acts on the epithelium together with adaptive immune signals, inducing epithelial secretion of IL-25, which represses ILC secretion of IL-22, and thus IL-22-induced AMPs. This equilibrium in the healthy mucosa is abrogated by epithelial insults, leading to increased IL-22 and activation of antimicrobial programs[40].

Microbial populations and ligands can have pro-inflammatory or anti-inflammatory effects. In mice, SFB promote T_H17 differentiation and IgA production, whereas *Clostridium* clusters IV and XIVa and parasite-secreted proteins such as *Heligmosomoides polygyrus* excretory-secretory antigen promote T_{reg}-cell differentiation[94–97]. Interestingly, patients with IBD show reduced representation of *Clostridium* clusters IV and XIVa, indicating one way in which anti-inflammatory T_{reg}-cell effects might be diminished, leading to a predisposition to inflammation[98]. The common constituent of normal human microflora *Bacteroides fragilis* produces polysaccharide A, which suppresses IL-17 production and promotes the activity of IL-10-producing $CD4^+$ T cells in mice[99]. The effects of microbial ligands and metabolites on adaptive immune function are exemplified by bacterial DNA signalling through TLR9 to limit T_{reg}-cell differentiation and promote intestinal immune responses to oral infection, and by bacterial ATP promoting T_H17 differentiation. Thus, the microflora shapes development and function of the mucosal immune system in a tightly correlated manner. Immune stimulatory effects of the microbiota are important to promote an effective response against potential pathogens, although dysregulated interactions, which might arise from perturbations in host, microbial or environmental factors, could lead to a loss of tolerance and promote intestinal inflammation.

Most of the observations detailing the mechanisms of microbe–host interactions have been made in mice, and correlations in humans remain to be defined. Microbes associated with human IBD include *Faecalibacterium prausnitzii*, adherent-invasive *E. coli*, invasive *Fusobacterium nucleatum* and mucolytic bacteria such as *Ruminococcus gnavus* and *Ruminococcus torques.* Reduced levels of *F. prausnitzii* in resected ileal mucosa from patients with Crohn's disease are associated with increased risk of endoscopic recurrence; *F. prausnitzii* stimulates IL-10 production in peripheral blood mononuclear cells, which may account at least in part for this protective effect[100]. Recent studies suggest that adherent-invasive *E. coli* exploits host defects in phagocytosis and autophagy arising from Crohn's-disease-related polymorphisms to promote chronic inflammation in the susceptible host[16]. Patients with IBD have a compromised mucus layer and an epithelial surface that is densely coated with bacteria; the abundant presence of *Ruminococcus* strains in IBD mucosa raises the possibility that such microbes may contribute to the barrier defect observed in IBD, although whether their presence is causal or correlative remains unclear.

These findings show that the composition of the microbiota and its interaction with the host are emerging as underappreciated sources of gene–environment interactions and are crucial to understanding the context of IBD. For example, alterations in the microflora community structure, as might occur in the context of antibiotic therapy or infectious colitis, can promote the development of IBD or trigger disease flares in patients with IBD. Identifying the factors that shape microbial community structure and function within an individual and that influence its restoration after perturbations will be key to understanding IBD pathogenesis. Obtaining such knowledge will require identifying associations between microbiome and human genetic studies at the very least.

Future perspectives

GWAS and next-generation sequencing technologies have provided insight into genetic definitions of host susceptibility. GWAS have unequivocally identified numerous genomic regions containing IBD-risk factors, showing several features of the genetic architecture of Crohn's disease and ulcerative colitis. First, IBD risk involves multigenic contributions, each with a relatively modest effect size. Second, genetic contributions to ulcerative colitis and Crohn's disease overlap, suggesting shared mechanistic features. Third, within Crohn's disease and ulcerative colitis, different clusters of risk loci are emerging, suggesting that these disease processes may comprise distinct pathological subsets beyond Crohn's disease versus ulcerative colitis. Accordingly, there is a need to define clinically relevant parameters that might help to classify Crohn's disease and ulcerative colitis further, including early-onset disease, stricturing disease, slow progressors, frequency of flares and response to therapeutics. Furthermore, given the importance of environmental factors in IBD risk, studies aimed at defining contributory environmental factors are greatly needed. Relevant approaches might include establishing prospective inception cohorts or following healthy, high-risk individuals, such as those with an affected first-degree relative.

An important adjunctive approach to GWAS is identifying rare variants, which frequently show larger effect sizes. The search for rare variants will help to prioritize the probable causal gene(s) within a locus (or loci) for experimental validation, identify disease-relevant pathways, and possibly identify domains important for protein function by leveraging natural mutations as a large forward genetic screen. Identifying and validating causal genes and assembling them into molecular pathways and cellular networks will require the use of patient samples and will considerably empower clinically relevant hypotheses. Given the diverse mechanisms that seem to participate in IBD, it will also become increasingly important to associate and stratify '-omic' measurements of RNA, protein, small molecules, chemical DNA modifications and gut microbiota according to patient genotypes.

There is a clear need to generate quantitative and qualitative expression maps of allelic variants. This notion is reinforced by the many polymorphisms implicating gene deserts, which probably contain regulatory elements. Furthermore, alternative splicing is a major contributor to the diversity of our transcriptome and its relevance to IBD has already been demonstrated by findings in *IL23R*.

The gut has many tiers of defence against incursion by luminal microbes, including the epithelial barrier, and the innate and adaptive immune responses. These components are all tightly interrelated, and disease requires breakdown at several checkpoints. Generating models to systematically analyse the defects arising from genetic variants associated with IBD is crucial. However, these variants may show the disease-relevant defect under select conditions, such as high bacterial load found in the colon, and the accompanying cytokine milieu.

Viral infections are common, and key studies highlight their potential to exert important immune modulatory effects. Acute and/or chronic viral infections could interact with host-susceptibility factors in a manner that leaves either the cell or the cellular milieu poised to promote pathological intestinal inflammation after subsequent triggering events. Notably, these studies highlight the need to characterize all microbial constituents (viral, fungal, parasitic and bacterial) in the context of IBD. Other tools need to be developed to study the microbiota at the level of species, geographical location, genetic variations, transcriptional dynamics, as well as changes to proteins and metabolites. Indeed, bacterial metabolites are principal mediators of interactions between microbial species, as well as between microbe and host, as exemplified by SCFAs. Studies to identify bioactive metabolites and other small molecules may thus have diagnostic and therapeutic potential.

An important goal is to combine these various facets to understand how genetic traits are integrated and propagated through physiological networks in the context of interactions with other genes, cells, microbes and environmental stimuli to control intestinal homeostasis. Genetic studies are already used to predict sensitivity to IBD therapies such as 6-mercaptopurine and may also be useful in predicting responses to biological therapies. Combining the different aspects of IBD pathophysiology may allow us to develop a more holistic understanding of the disease, thus promoting advances in diagnostics and therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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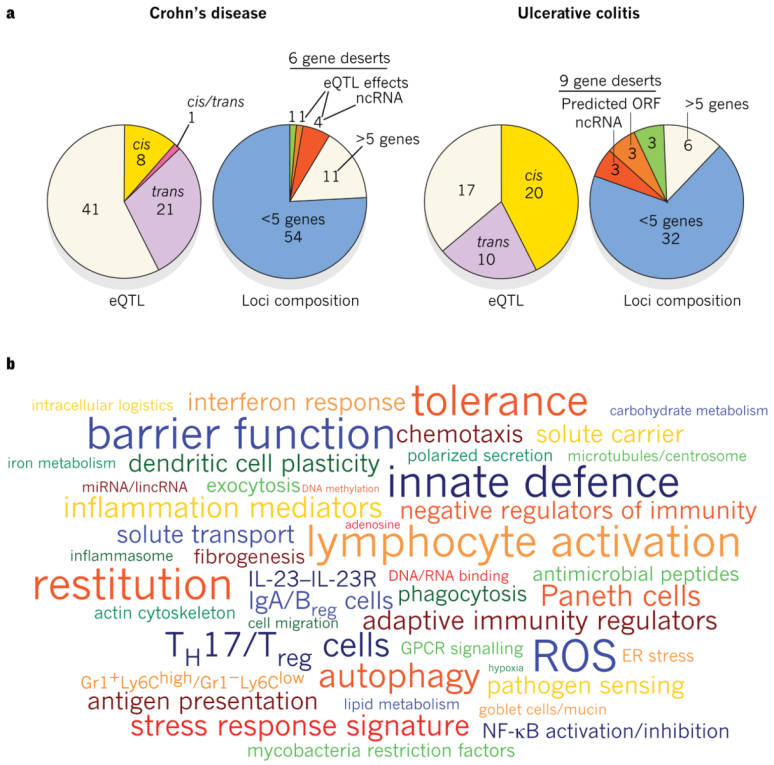

Figure 1. Genetic architecture of IBD-linked susceptibility loci

a, GWAS have identified 71 risk loci in Crohn's disease and 47 risk loci in ulcerative colitis (*P* value of association < 5×10^{-8}). Of these, 28 risk loci exhibit shared associations (defined as $P < 5\times10^{-8}$ for either Crohn's disease or ulcerative colitis, and $P < 1\times10^{-4}$ for the other form of IBD). Approximately half of the loci implicated in Crohn's disease and ulcerative colitis are associated with *cis*- and/or *trans*-expression quantitative trait loci (eQTL) effects (left panels). Genes whose expression are affected by these variants could also be involved in IBD pathogenesis. The loci composition (right panels) shows the number of genes that either lie within or segregate in linkage disequilibrium with IBD-implicated loci (coefficient of correlation $r^2 > 0.8$). These loci are structurally heterogeneous, and are associated with widely ranging numbers of genes. Loci not associated with any genes, known as gene deserts, frequently contain non-coding transcripts or predicted open reading frames (ORFs), and can be associated with *trans*-eQTL effects. **b**, Recurring terms illustrating biological processes implicated by at least three genes represented in IBD loci; font sizes are proportional to the number of genes associated with each respective process. B_{reg} cells, B regulatory cells; ER, endoplasmic reticulum; GPCR, G-protein-coupled receptor; IL, interleukin; lincRNA, large intervening non-coding RNA; miRNA, microRNA; ncRNA, non-coding RNA; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; T_H17 cells, T helper 17 cells; T_{reg} cells, T regulatory cells.

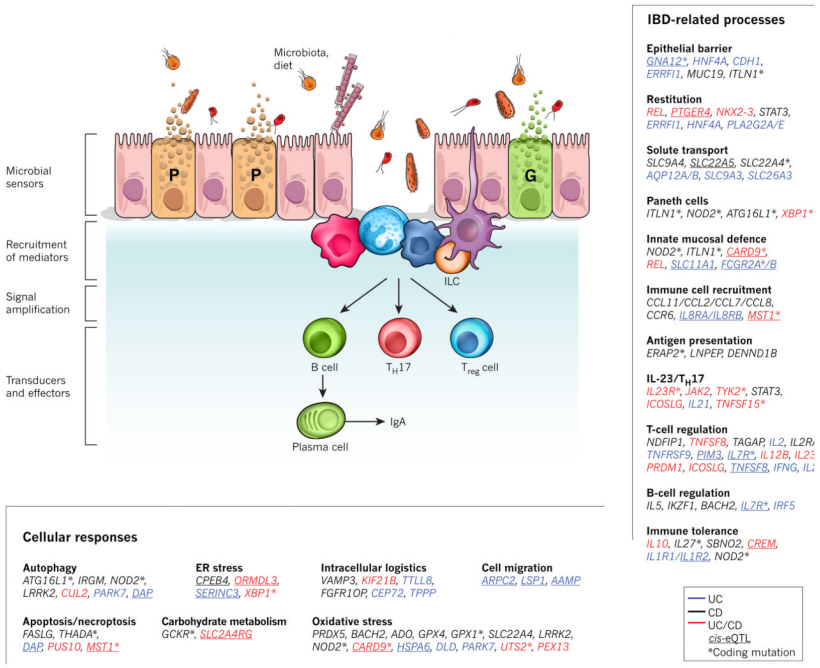

Figure 2. A model for IBD pathways based on GWAS

Intestinal homeostasis involves the coordinated actions of epithelial, innate and adaptive immune cells. Barrier permeability permits microbial incursion, which is detected by the innate immune system, which then orchestrates appropriate tolerogenic, inflammatory and restitutive responses in part by releasing extracellular mediators that recruit other cellular components, including adaptive immune cells. Genetic variants, the microbiota and immune factors affect the balance of these signals. Genes in linkage disequilibrium ($r^2 > 0.8$) with IBD-associated single nucleotide polymorphisms (SNPs) were manually curated and classified according to their function(s) in the context of intestinal homeostasis and immunity. Text colour indicates whether the genes are linked to risk loci associated with Crohn's disease (CD; black), ulcerative colitis (UC; blue) or both (red). Asterisk denotes corresponding coding mutations; *cis*-eQTL effects are underlined. G, goblet cell; P, Paneth cell.

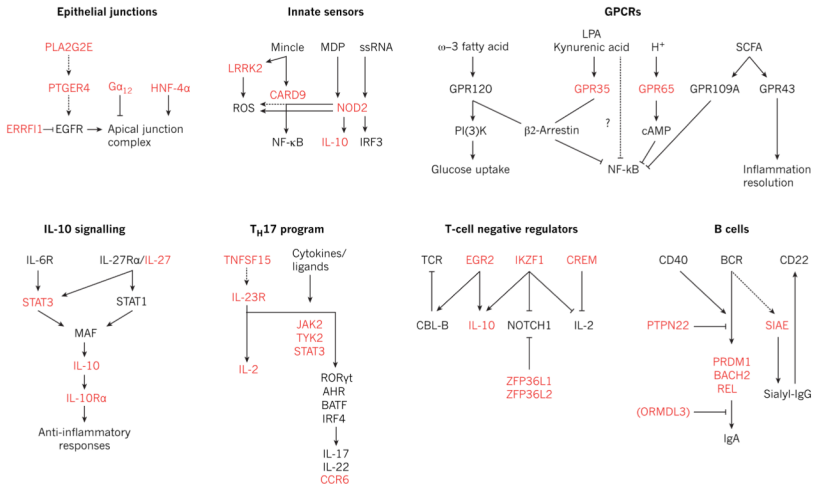

Figure 3. Genetic variants in IBD signalling modules

Schematic of selected signalling pathways involved in the maintenance of intestinal homeostasis, including epithelial junctional complex assembly, innate immune recognition of pathogen-associated motifs, GPCRs and immune defence, anti-inflammatory interleukin-10 (IL-10) signalling, T_H17-cell differentiation, inhibitory pathways in lymphocyte signalling, and B-cell activation and IgA antibody responses. Proteins encoded by genes identified as being in linkage disequilibrium with IBD-risk SNPs ($r^2 > 0.8$) are highlighted in red. BCR, B-cell receptor; cAMP, cyclic AMP; EGFR, epidermal growth factor receptor; ERRFI1, ERBB receptor feedback inhibitor 1; $G\alpha_{12}$, G protein subunit α_{12}; GPCR, G-protein-coupled receptor; HNF-4α, hepatocyte nuclear factor-4α; LPA, lysophosphatidic acid; MDP, muramyl dipeptide; NF-κB, nuclear factor-κB; PI(3)K, phosphatidylinositol-3-OH kinase; PLA2G2E, phospholipase A_2, group IIE; PTGER4, prostaglandin E receptor 4; SCFA, short-chain fatty acid; SIAE, sialic acid acetylesterase; ssRNA, single-stranded RNA; TCR, T-cell receptor.

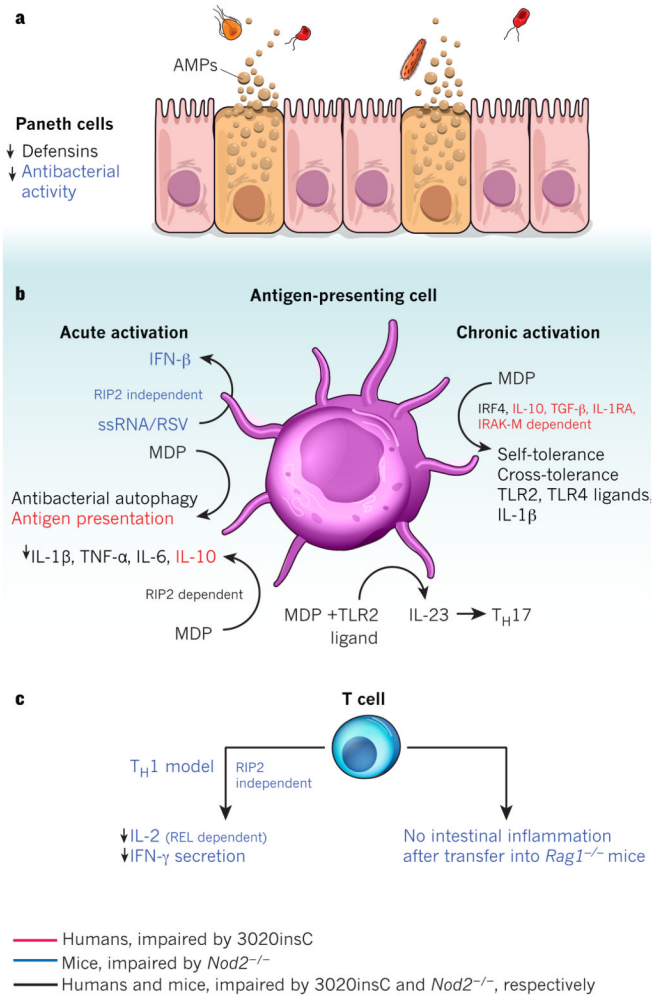

Figure 4. Cell-intrinsic functions of NOD2

NOD2 is activated by the bacterial peptidoglycan muramyl dipeptide (MDP). Cell-specific NOD2 functions are shown, distinguishing between those functions impaired in cells from humans with the Crohn's-disease-associated mutation 3020insC (red), from *Nod2*-deficient mice (blue), or from both (black). **a**, In Paneth cells, *Nod2* deficiency leads to attenuated antibacterial activity in the intestinal crypts and decreased expression of α-defensin 4 (encoded by *Defcr4*, also known as *Defa4*) and α-defensin-related sequence 10 (DEFCR-RS10, also known as DEFA-RS10). **b**, MDP-stimulated release of pro-inflammatory NF-κB-dependent cytokines (such as IL-1β, TNF-α and IL-6), as well as secretion of IL-23 (which promotes T_H17 differentiation) after co-stimulation with MDP and TLR2 ligands, is decreased in antigen-presenting cells from *Nod2*-deficient mice or 3020insC human donors. MDP stimulation also leads to NOD2-activated autophagy and antigen presentation. In mice, the activation of antigen-presenting cells by ssRNA or respiratory syncytial virus (RSV) stimulates secretion of type I interferon (IFN-β) in a NOD2-dependent, receptor-interacting protein-2 (RIP2)-independent fashion. In contrast to the pro-inflammatory effects, chronic NOD2 activation (right) by MDP induces both self-tolerance and cross-tolerance to IL-1β, and TLR2 and TLR4 ligands. This is dependent on IRF4 in mice and humans, and also on IL-10, TGF-β, IL-1RA and IL-1R-associated kinase M (IRAK-M) in humans. MDP-induced tolerance is lost in *Nod2*-deficient mice and in patients with the 3020insC variant. NOD2-dependent release of IL-10 after MDP stimulation has been demonstrated to be specific to

humans and is impaired in 3020insC cells. MDP-stimulated release of several cytokines, including IL-10, IL-1β, TNF-α and IL-6, is dependent on RIP2. **c**, In mice, NOD2 mediates IFN-γ secretion and REL-dependent IL-2 production in T cells in response to *Toxoplasma gondii* infection. Also, *Nod2* deficiency attenuates the ability of T cells to cause experimental colitis after transfer into *Rag1*-deficient hosts.

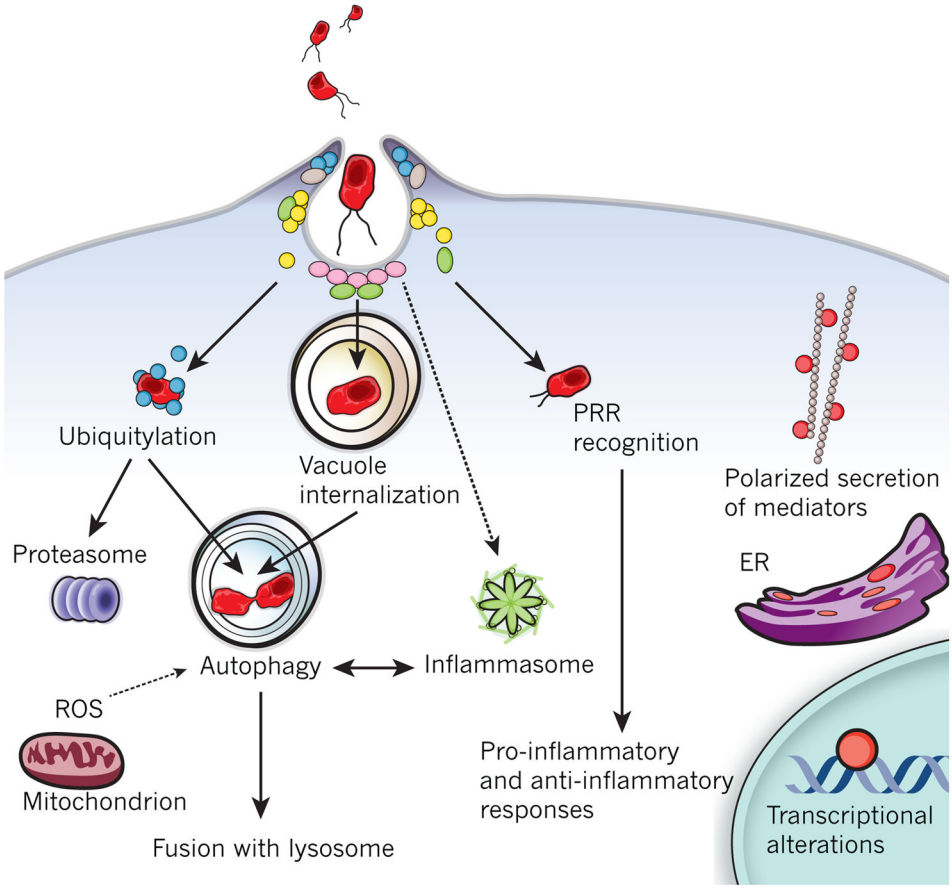

Figure 5. Intracellular defence programs in microbial recognition

Host cells have evolved processes by which they restrict the availability of intracellular permissive niches to microbes. Microbial recognition by PRRs, such as NOD proteins and TLRs, activates key immediate host programs, leading to polarized secretion of pro-inflammatory mediators (directed to either the luminal or basolateral surface). Bacteria can either be maintained in subcellular compartments such as microbe-containing vacuoles, or escape into the cytoplasm, where they can be ubiquitylated and targeted for degradation. Both subsets can be targeted by the autophagy pathway, which is also regulated by other host defence mechanisms such as oxidative stress and inflammasome activation.