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Pathway Paradigms Revealed from the Genetics of Inflammatory Bowel Disease

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Preface

Inflammatory bowel disease (IBD) is a complex genetic disease that is instigated and amplified by the confluence of multiple genetic and environmental variables that perturb the immune-microbiome axis. The challenge of dissecting pathological mechanisms underlying IBD has spawned the development of transformative approaches in human genetics and functional genomics. Here, we describe IBD as a model disease in the context of leveraging human genetics to dissect cellular and molecular pathway interactions that control homeostasis of the mucosal immune system. Finally, we synthesize emerging insights from multiple experimental approaches into pathway paradigms and discuss future prospects for disease subtype classification and therapeutic intervention.

Introduction

The contemporary view of inflammatory bowel disease (IBD) has evolved rapidly as a result of recent advancements in human genetics, mucosal immunology, and microbiome research. IBD is a chronic relapsing and remitting disease associated with dysregulation of the mucosal immune system and the commensal ecosystem. As such, IBD is regarded as a model disease that exemplifies the complexities of interactions between genetic, immune and environmental variables that coordinately impact disease. IBD genetics has benefited significantly from studying common variants in large cohorts of well-phenotyped patients and rare variants in cases associated with Mendelian inheritance. Functional studies inspired by IBD genetics helped to uncover fundamental mechanisms of immunity, host-microbe interactions, and offer actionable insights into therapeutic innovation. Accordingly, the last

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decade of IBD research exposed genetic vulnerabilities in an expansive interconnected network of host pathways that intimately interface with the microbiome. This work has helped to shape the emerging view of IBD as a system-level perturbation of the mucosal immune system and commensal ecosystem. This network dyshomeostasis model defies simple cause and effect explanations of IBD triggers; rather, it invokes many environmental variables acting cumulatively over time on the backdrop of many genetic variants to explain the evolution of the pathological relationship between host and microbiome. Below, we highlight significant advancements in our understanding of IBD pathogenesis that provide novel insights into human biology with relevance across the spectrum of health and disease.

Clinical Features of IBD.

IBD is viewed as two subtypes, ulcerative colitis (UC) and Crohn's disease (CD), with UC affecting the colon and CD affecting any region of the gastrointestinal tract, but primarily the terminal ileum of the small intestine[1]. IBD onset typically occurs in early adulthood, although it can occur at any age and is increasing in prevalence at all ages, including in early onset and geriatric populations. IBD is driven by genetics and environment, such that dysregulated mucosal immune function is associated with a dysbiotic commensal microbiome that coordinately drives a waxing and waning pathological inflammatory cycle. Although IBD has historically been considered a disease of the West, incidences in the Eastern hemisphere are increasing dramatically and follow geographical patterns of industrialization/westernization[2].

Heterogeneity of Disease.

Accumulating evidence indicates that IBD is more heterogeneous than the traditional UC/CD dichotomy. Thus, IBD likely comprises many disease subtypes that are distinguishable based on the natural history of disease, response to treatment, and distinct genetic risk factors. Clear distinctions based on molecular phenotypes remain elusive. With bourgeoning clinical datasets comprising genotype data, molecular diagnostics, and metagenomics, the field is poised to define a clinically informative framework for classifying and treating IBD subtypes[3]. New biomarkers have emerged from similar efforts and prognostic markers in blood have been identified to classify IBD patients based on risk of frequent relapse and complicated clinical outcomes. In particular, a CD8 T cell exhaustion gene expression signature predicts positive outcomes in IBD and autoimmunity[4].

Clinical studies provide novel insights into heterogeneity of disease at the level of pathogenesis and treatment response rates. Accordingly, inception cohorts offer a unique view of IBD at the time of diagnosis, prior to therapeutic intervention, and enable longitudinal monitoring of disease progression[5]. It is important to note the inherent challenges and potential power of patient-driven research and reverse translation of clinical observations into hypotheses for validation in the laboratory. Indeed, the field is in early stages of translating gene expression signatures and multi'omic data into a tangible understanding of disease. Nevertheless, these data function as a snapshot of disease that can be mined and integrated with other data, including genetics and functional biology experiments. For example, expression of genes within the IL-7R pathway were elevated prior to treatment in patients that subsequently failed anti-TNF[6]. In experimental models,

IL-7R signaling induced expression of α4β7 integrin and imprinted gut homing ability upon T cells. In humanized mice, IL-7R blockade reduced T cell homing to the gut and subsequent intestinal inflammation[6]. These studies shed light on why subsets of patients may respond to treatment while others lose responsiveness or fail to respond initially. Given that a significant percentage of patients do not respond to anti-TNF and other biologics, there is a pressing need to identify new therapeutic mechanisms of action.

Intestinal Complications and Learning Opportunities.

IBD pathology escalates and often leads to the development of heterogeneous comorbidities and other complications that shed light on mechanisms underlying intestinal homeostasis. In some cases, colectomy in UC patients can evolve into a CD-like disease associated with ileal pouch inflammation (pouchitis), demonstrating a level of plasticity in disease progression that is poorly understood. Additionally, UC can be associated with complications affecting the gut-liver axis such as primary sclerosing cholangitis (PSC). PSC is a chronic idiopathic cholangiopathy that can progress to cirrhosis and liver disease and is often associated with liver, gallbladder, or colon carcinogenesis. Thus, the burden of IBD over time is particularly evident in the elevated risk of cancer associated with both CD and UC. Conversely, intestinal pathologies can arise as comorbidities associated with other clinical contexts. For example, colitis associated with checkpoint inhibition tumor immunotherapy highlights the importance of T cell coinhibitory pathways in maintaining intestinal tolerance[7]. Furthermore, IL-17 has emerged as a surprising arbiter of intestinal homeostasis based on clinical observations of exacerbated IBD symptoms in psoriasis patients treated with biologics that inhibit the IL-17 pathway[8]. Taken together, studying intestinal complications arising from IBD and other diseases can reveal unexpected mechanisms of immunoregulation.

Genetics of IBD

Common Variant Association Studies and Fine Mapping Disease Traits.

The post-human genome era rapidly ushered in efforts to map the genetic heterogeneity of human populations with the objective of functionally assigning genotype-phenotype relationships, particularly in the context of complex genetic diseases. Although GWAS have been remarkably successful at implicating IBD risk loci, identifying the causal genes and variants within these loci requires additional approaches (Figure 1). GWAS have been empowered by expanding arrays to allow dense genotyping of key immune-related loci[9]. As the number of IBD cases in GWAS increased, so has the power to identify additional risk-associated loci. Ongoing efforts to incorporate diverse ancestry in GWAS shows promise in capturing global genetic diversity that contributes to disease risk, although this may require genotyping strategies that capture genetic variation beyond what is typically captured in European-derived ancestry[10]. Trans-ancestry association studies of IBD have identified differential risk associations for *NOD2* and *TNFSF15* wherein the former dominates in European and the latter in East Asian populations[10]. The most recent integrative analysis of IBD GWAS analyzed nearly 60,000 subjects (including over 25,000 IBD cases) and identified approximately 240 loci statistically associated with risk of developing IBD[11]. Fine-mapping GWAS studies have helped to refine risk loci to implicate specific causal

SNPs associated with IBD[12,13]. Although this approach is powerful, it has limitations for risk haplotypes that comprise many noncoding SNPs that exist in linkage disequilibrium and cooperatively function to impact gene expression through epigenetic mechanisms[14,15]. With a growing number of loci associated with IBD risk and progression, the field is poised to convert these associations into causal disease mechanisms[16].

Exome Sequencing Identifies Risk and Protective Variants.

Genotyping SNPs in GWAS assigns a binary risk or non-risk phenotype to haplotypes spanning genomic loci comprised of SNPs across multiple genes and intergenic regions. In contrast, exome sequencing is not constrained by genotyping known variants, and is powered for discovery of rare coding variants that act independently of common haplotypes and often influence disease risk with larger effect sizes. Searching for rare causal variants by exome sequencing is constrained by the requirement for large cohorts. One way around this potential limitation is to study populations that have been subject to a founder effect, or genetic bottleneck, in which rare alleles and their associated phenotypes may be enriched[17–19]. One example is the Ashkenazi Jewish (AJ) population, in which the incidence of CD is 2–4 times higher than non-Jewish (NJ) European populations[17]. Exome sequencing studies comparing AJ vs NJ European populations revealed novel coding variants in GWAS loci and in novel genes that may explain the increased incidence of CD in the AJ population[17]. CD risk in the AJ population is enriched in genes related to autophagy, whereas in the NJ population, it is enriched for genes involved in the IL-23/17 pathway[17]. Genetic studies in cohorts of different ethnicities hold great potential for identifying the heterogeneity underlying genetic risk factors and suggest the existence of discrete disease subtypes potentially driven by distinct mechanisms.

Exome sequencing has identified multiple independent coding variants in genes that collectively represent an allelic series associated with a spectrum of phenotypes, ranging from risk to protection from disease. For example, exome sequencing identified risk and protective variants comprising allelic series in several genes including CARD9[20,21], IL23R[20], and RNF186[19,21]. These findings highlight the importance of innate microbial sensing pathways, cytokine networks, and barrier function in IBD risk. Additional functional studies are required to reveal the mechanistic basis of risk versus protection, and as discussed below, these efforts are important for guiding the development of therapeutic strategies to inhibit risk-related mechanisms or mimic protective mechanisms (Figure 1).

Mendelian Genetics.

Genome sequencing has revolutionized mapping of genetic variants to monogenic traits in the context of Mendelian disorders. This is particularly true in cases of primary immunodeficiencies associated with intestinal manifestations and in very early onset IBD (VEOIBD), which typically occurs in kindred prior to the age of six. The discovery of causal genes and variants associated with severe intestinal pathologies uncovered unique mechanisms controlling intestinal homeostasis. One of the first genes associated with VEOIBD was *IL10RA*, in which severe hypomorphic alleles reduced IL-10 signaling, resulting in impaired tolerance[22]. IL-10R signaling in macrophages is essential for limiting intestinal inflammation[23–25]. Similarly, GWAS studies identified noncoding SNPs near *IL10*

and a weaker signal in the *IL10RA* locus associated with adult onset IBD. Importantly, Mendelian disorders uncovered novel genes and pathways associated with intestinal pathology not detected in GWAS (Figure 1). To date, more than 350 primary immunopathologies have been identified and linked with distinct molecular etiologies, roughly one third of which are associated with gastrointestinal symptoms[26]. These immunopathologies span the phenotypic spectrum from immunodeficiencies to hyperinflammatory disorders. For example, lymphoproliferative disorders associated with deficiencies in cell death regulating genes, such as Caspase 8 and *XIAP*, or genes involved in T cell regulatory function such as *FOXP3* or *CTLA4*[26] typically elicit intestinal inflammation. On the opposite end of the spectrum, mutations linked to immunodeficiencies revealed insights into gut mucosal immunity. Defective TCR repertoire selection was observed in kindred bearing a novel mutation in *MALT1* that impaired NFkB signaling[27]. Additionally, intestinal manifestations occur in innate immunodeficiencies such as chronic granulomatous disease (CGD), which is associated with mutations in *CYBB*[28]. Similarly, the lysosomal storage disease Niemann-Pick disease type C1 manifests with CD like symptoms due to impaired antibacterial activity[29]. Taken together, emerging insights suggest that IBD is associated with hyperinflammatory conditions related to cytokine imbalance and also immunodeficiencies related to defective antimicrobial responses. Immunodeficiencies may lead to an unhealthy relationship with the microbiome, thus offering a potential explanation for why these pathologies target the intestine.

A number of genetic variants have been linked with Mendelian diseases associated with intestinal pathologies that directly impact epithelial function[30]. In particular, regulators of epithelial polarity such as *TTC7A* are associated with VEOIBD. *TTC7A* is thought to function as a critical component of the PI4KIIIα complex to maintain epithelial apico-basal polarity[31]. Other examples highlight genes controlling epithelial barrier functions, such as the epithelial adhesion molecule *EPCAM* and *SPINT2*[32], which are associated with congenital tufting enteropathy and congenital sodium diarrhea, respectively. In fact, mechanisms controlling intestinal electrolyte balance are important genetic vulnerabilities for intestinal inflammation. Familial *GUCY2C* diarrhea syndrome, which is linked to a high prevalence of IBD, is associated with gain of function variants that induce intestinal secretion of NaCl and water through CFTR and inhibition of sodium hydrogen exchanger 3 (NHE3)[33]. *GUCY2C* encodes an epithelial receptor that recognizes endogenous ligands (uroguanylin and guanylin) and induces production of cyclic guanosine monophosphate (cGMP), which regulates CFTR through protein kinase GII activation. Taken together, these findings implicate novel genes that regulate pathways, such as electrolyte balance, epithelial polarity, and barrier function.

Functional Genomics.

With a rapidly expanding number of genetic associations statistically linked with IBD and intestinal pathologies, the field is moving to convert these insights into a mechanistic understanding of disease risk or pathological mechanisms. Complicating matters, genetic associations occur in a background of thousands of genetic variants per individual that collectively interact with environmental variables to impact disease. Traditionally, implicating a gene or variant as causal with respect to a phenotype involves gene knock-out

studies to demonstrate the requirement for a gene, or alternatively, knock-in to determine sufficiency for a variant to induce a phenotype (Figure 1). Knock-out or knock-down screening strategies have been employed to demonstrate the requirement for candidate IBD risk genes in pathways such as autophagy[34], antimicrobial function[35], Th17 development[36], and inflammatory cytokine production[37,38]. CRISPR- based approaches have also been developed to functionally map noncoding regions of the genome, such as enhancers, that are associated with diseases such as IBD[39]. CRISPR-mediated genome editing technologies enable functional characterization of coding variants implicated in IBD in isogenic cells and mouse models that differ from their corresponding controls only in the introduced variant. These approaches interrogate the phenotype of a single coding variant, and emerging CRISPR methodologies using base editing technology suggest the possibility of phenotyping many IBD risk variants in parallel, in screening mode.

Mouse models have been a mainstay for modeling intestinal inflammation, while mechanistic *in vitro* studies have benefitted from advancements in organoid technology. Organoid models recapitulate key intestinal epithelial features and can be engineered using CRISPR technology to functionally characterize disease-associated genes[40]. These organoid systems have been exploited to define gene function in epithelial-intrinsic cellular processes and also to dissect crosstalk between immune and epithelial lineages[41]. Increasingly sophisticated coculture systems are being developed to mimic intestinal biology *in vitro* in gut-on-a-chip platforms that incorporate multiple cell types, microbial communities, and physiological conditions such as oxygen gradients and forces associated with fluid flow[42]. These model systems hold great potential for identifying functional roles for IBD risk genes and variants.

Single Cell Technology and the Disease Atlas.

As *in vitro* cellular models become more sophisticated and capable, new technologies in single cell genomics offer an unprecedented view of the disease process in patients. Single cell transcriptomics allows for an unbiased census of cell lineages and their functional states in health and disease. Perhaps more importantly, single cell studies are powered to deconvolute cellular interactions and pathway crosstalk underlying the pathophysiology of disease. Several recent studies performed single cell transcriptomics on intestinal biopsies derived from UC patients[43–45], CD patients[46], and mouse models of enteric infection[47]. Accordingly, a cellular atlas was constructed from paired biopsies derived from UC patients and healthy controls resulting in identification of 51 cell types/states based on transcriptomic profiles[45]. These profiles capture important cellular lineages and biological functions impacting IBD and intestinal homeostasis (Figure 2). During inflammation, the cellular composition of the mucosa changes, with a notable influx of inflammatory monocytes and differentiation of inflammatory fibroblasts[45]. Modeling intercellular communication by mapping expression of receptor-ligand pairs revealed a potential functional link between inflammatory monocytes, which produce the IL-6 family cytokine OSM and inflammation-associated fibroblasts, which express the receptor heterodimer OSMR-IL6ST[45]. In fact, this myeloid cell-derived OSM circuit with fibroblasts induces expression of inflammatory chemokines and is implicated in severe IBD associated with resistance to anti-TNF therapy[48]. Integrating IBD GWAS and disease atlases can be leveraged to identify precise

cell type(s) in which candidate genes are expressed, whether disease status correlates with their transcriptional regulation, or if they are transcriptionally coregulated within functionally related gene modules that hint at mechanisms of action (Figure 2).

Regulation of gene expression is cell type-specific and context-dependent. Thus, quantification at single cell resolution is a powerful approach for determining how common genetic variants associated with IBD impact gene expression and cellular function. Future studies leveraging single cell RNAseq for eQTL studies in healthy versus inflamed intestinal biopsies may provide a deeper understanding of the impact of genetic variation at the population level on gene expression in IBD risk and progression. Thus, gene expression datasets derived from intestinal tissues can be integrated with IBD genetics to establish gene-trait associations. In this context, single cell transcriptomic datasets comprising the disease atlas offer a high-resolution view of individual cellular transcriptomes. Building the disease atlas as a reference dataset yields a powerful tool for iteratively modeling, testing, and revising mechanistic models of disease in experimental settings, animal models, and clinical studies.

Pathways

IBD as a Model Disease.

Studies of IBD genetics have implicated genes and pathways underlying inflammatory pathology. Many core cytokine pathways implicated in IBD GWAS overlap amongst inflammatory diseases and autoimmunity, while IBD genetics provides a unique view of pathways that control mucosal immunity[49]. As such, this core set of pathways represents vulnerabilities to disease development that suggest potential opportunities for intervention (Figure 3).

The Epithelial Barrier and Dynamic Remodeling of Junctional Complexes.

The gut mucosa comprises specialized cell types that functionally interconnect host physiological systems with extrinsic commensal communities, pathogens, metabolites, and dietary factors. While epithelial cells maintain a physical barrier separating the host from its environment, they simultaneously interact with their environment to relay information throughout the body and coordinate an appropriate host response. Specialized intestinal epithelial cell lineages derive from ISCs positioned at the base of invaginated crypt structures[47]. The self-renewing stem cell compartment is located within a unique niche that is supported by signals from the microbiota and growth factors derived from stroma and accessory epithelial cells. Chronic inflammation associated with IBD damages crypts and impairs stem cell reprogramming during epithelial restitution. Insights from IBD genetics implicate the aryl hydrocarbon receptor (*AHR*) as a critical sensor of the luminal microenvironment capable of protecting the stem cell niche from inflammatory damage[50]. This niche is also regulated by Paneth cells in the small intestine, which secrete growth factors that promote ISC regeneration during restitution or inhibit it during chronic inflammation[51,52]. In addition, Paneth cells participate in innate immunity by producing antimicrobial peptides that protect the crypt from microbial infestation. Similarly, goblet cells also link innate immunity with barrier function by producing and secreting a protective

mucus layer on the luminal surface of the intestinal epithelium. Goblet cells and Paneth cells uniquely express *ITLN1*, an IBD risk gene encoding a lectin thought to bind carbohydrate moieties on the surface of microbiota and promote host innate immunity[45].

The most abundant intestinal epithelial cell type is the absorptive enterocyte, which helps establish the physical barrier of the epithelium, mediate nutrient and water uptake, and possesses innate pathogen-sensing capabilities. In active CD, enterocytes exhibit morphological defects in brush border and microvillus structure consistent with impaired nutrient absorption and barrier function[53]. Additionally, several IBD risk genes are enriched in expression in enterocytes and contribute to lineage specification (*HNF4A*), junctional integrity (*C1orf106*), and innate immunity (*GSDMB*)[45]. Specialized epithelial cells also contribute to adaptive immunity. Microfold cells (M cells), are directly apposed to secondary lymphoid structures in the mucosa, and transport luminal antigens to lymphoid follicles. M cells expand in number in inflamed regions of the colon of UC patients[45] and perform critical functions in immune-microbiome homeostasis[54]. Similarly, Tuft cells interface with the microbiome by acting as chemosensory sentinels that detect pathogens and toxins and subsequently elicit type 2 immune responses through the production of IL-25[55]. Another important class of sentinels in the epithelium are Enteroendocrine cells, which function as sensors and effectors that produce hormones to coordinate with the enteric nervous system (ENS) and other digestive organs. Subsets of regionally-specific Enteroendocrine cells are characterized by the unique repertoires of hormones they produce[56,57] and how their functions change during inflammation[47]. For example, serotonin-producing Enteroendocrine cells sense changes in the microbiome milieu through G protein-coupled receptors (GPCRs) that initiate serotonin secretion and subsequent engagement of serotonergic neurons, which control gut motility[58].

Genetic studies of IBD have identified novel regulators of diverse epithelial functions. In particular, genes controlling barrier integrity, such as *C1orf106*, *RNF186*, and *HNF4A* represent key risk factors for UC[11] (Figure 3). *C1orf106* was first implicated in UC GWAS, and exome sequencing subsequently identified a coding variant associated with UC risk[20]. In functional studies, *C1orf106* functions as an adaptor that regulates ubiquitination and degradation of cytohesin proteins, which are guanine nucleotide exchange factors for the small G protein ARF6[59]. ARF6 plays a critical role in endocytic recycling of cell surface receptors, including cadherin proteins, which form adherens junctions that physically link epithelial cells together in monolayers[59]. The IBD risk variant of C1orf106 Y33F is associated with elevated levels of cytohesins, defective remodeling of adherens junctions, and subsequent impairment of barrier function, which is thought to sensitize to chronic microbiota-driven intestinal inflammation[59,60].

IBD GWAS identified another novel regulator of epithelial barrier integrity, the ubiquitin ligase *RNF186*[9]. Exome sequencing subsequently identified an allelic series of coding variants for RNF186, including the A64T missense variant associated with UC risk[21] and the R179X nonsense variant associated with protection from UC[19]. Rnf186-deficient mice and A64T knock-in mice exhibit increased intestinal permeability indicative of epithelial barrier dysfunction[61]. Rnf186 may regulate turnover of tight junctions by ubiquitinating occludin[61], although it is likely that it regulates additional epithelial cell functions such as ER stress

responses[62]. While Rnf186 A64T is associated with epithelial barrier dysfunction, it remains unclear how the protective variant R179X impacts intestinal epithelial biology. In humans, RNF186 R179X is associated with elevated serum creatinine[63], derived from the decomposition of creatine, which functions as an important cellular energy shuttle. Within epithelial cells, creatine transports high energy phosphates from the mitochondria to the apical surface of polarized epithelial cells, where ATPase myosin motor proteins regulate assembly of microvilli and contraction of the actomyosin belt to maintain dynamic barrier integrity. Thus, dissecting pathways that control intestinal barrier function may reveal novel targets for therapeutic development.

Innate Microbial Sensor Pathways.

The epithelial barrier sequesters commensal microorganisms from the host innate immune system, which is equipped with an arsenal of microbe-sensing mechanisms. *NOD2* was one of the first genes conclusively linked to IBD[12,20,64,65]. NOD2 functions as an intracellular cytosolic sensor of muramyl dipeptide (MDP), a component of peptidoglycan derived from bacterial cell walls. Engagement of NOD2 by MDP activates NFkB via RIPK2, and cooperates with inflammasomes to promote IL-1b secretion[66,67]. A mouse model for *Nod2* fs1007insC demonstrated impaired cytokine production in macrophages in response to MDP[68]. Separate studies suggest that CD-associated *NOD2* variants impair NFkB activation, indicating that attenuated NOD2 signaling may result in unproductive antibacterial responses leading to hyperinflammatory phenotypes. In fact, Nod2-deficient mice exhibited increased pathology in epithelial injury models, suggesting that impaired Nod2 signaling may allow inflammatory signaling to persist through alternative pathways[69], and/or that microbiome pathobionts can tip the balance towards chronic inflammation[70]. Specifically, Nod2-deficiency resulted in excess Nlrp3 microbiome-driven activation, and a small molecule inhibitor of Nlrp3 abrogated intestinal inflammation in Nod2-deficient mice[69]. Thus, *NOD2* functions in the intestine as a critical microbial sensor and inflammatory effector impinged upon by additional IBD risk genes, including *RIPK2*[11], *XIAP*[71,72], *TRIM22*[73], and *TNFAIP3*[74]. While variants in *NOD2* and *NOD2*-regulating pathways represent genetic vulnerabilities to intestinal pathology, they are also potential drug targets[75,76].

Like NOD2, CARD9 functions in microbial sensing pathways highlighted by IBD genetics[11] (Figure 3). As an adaptor protein, CARD9 is required for NFkB activation and cytokine production downstream of myeloid receptors that signal through immunoreceptor tyrosine-based activation motifs (ITAMs)[77]. In particular, CARD9 is required for inducing cytokine production in phagocytes after engagement of Dectin-1 by fungal ligands, which secondarily promotes Th17 immunity. Several loss of function *CARD9* alleles are associated with susceptibility to lethal fungal infections in humans, while in IBD, an allelic series was identified for *CARD9* that comprises risk and protective alleles. The missense variant CARD9 S12N is associated with IBD risk[20,21]. Conversely, a protective variant CARD9 IV11+1G>C (c.1434+1G>C)(rs141992399) disrupts mRNA splicing and is thought to result in exon skipping and a frameshift that truncates CARD9 prior to its C- terminal domain (CTD)[20,21] (Figure 3). A mouse model for Card9 S12N revealed an unexpected function for the risk variant in alveolar macrophages associated with increased cytokine production in

response to fungal ligands[78]. In CD patients, commensal fungi such as *Malassezia restricta* colonize the intestines of S12N carriers at higher rates compared to S12 patients, and this unusual colonization is proposed to amplify chronic inflammation associated with CD[79]. While the *CARD9* risk allele S12N is associated with enhanced inflammatory cytokine production in myeloid cells, the protective truncated CARD9 (deltaCTD) functions as a dominant negative and is associated with reduced cytokine production in dendritic cells after stimulation with fungal ligands[80]. Mechanistically, the truncated CARD9 deltaCTD protein is unable to physically interact with the ubiquitin ligase TRIM62[80]. In overexpression systems, TRIM62 is sufficient to ubiquitinate CARD9 at K125, and this post-translational modification is required for CARD9-mediated activation of NFkB downstream of fungal receptors[80]. Taken together, these findings suggest that mimicking the function of the protective IV11+1G>C allele by disrupting ubiquitination of CARD9 may represent a safe and effective therapeutic intervention in IBD[81].

Humoral Immunity and Antibodies in IBD.

Innate antimicrobial immune responses are complemented by functions of the adaptive immune system. B lymphocytes comprise a major arm of the mucosal immune system responsible for immunity to commensal microbes and pathogens. The mucosa is enriched in IgA antibodies that both promote and limit colonization by commensals to maintain intestinal homeostasis[82]. While IgA antibodies are considered to be neutralizing, other isotypes found in intestinal tissue can engage host cellular effector mechanisms such as the complement system and Fc receptors. For example, UC patients exhibit unusually high levels of commensal-reactive IgG in the colonic mucosa[83]. In turn, IgG-commensal immune complexes can engage Fc gamma receptors on macrophages and induce IL-1b and neutrophil-specific chemokines[83]. A missense coding variant in *FCGR2A* resulting in H131R substitution[9] is protective with respect to UC risk and reduces the affinity of Fc gamma receptor for immune complexes, thus attenuating macrophage inflammatory responses and subsequent type 17 immunity[83]. With a greater understanding of the molecular composition of the human intestinal microbiome[3], the field is poised to identify novel bioactives and antigens that impact host pathways in health and IBD[84].

Cellular Immunity and Lymphocytes.

Antigen presentation operates at the epicenter of immunity, linking phagocytosis and microbial killing with T cell activation and differentiation. Indeed, many of the phagocyte effector pathways highlighted by IBD genetics, and discussed herein, directly impact processing and presentation of antigenic peptides on MHCII for activation of conventional alpha/beta T cells (Figure 3). Fine mapping the human leukocyte antigen (HLA) locus directly implicated MHCII alleles in risk of developing CD or UC[85]. Moreover, MHCII alleles were identified in a GWAS for CD prognosis that strongly associate with severe clinical outcomes[86]. In addition, IBD risk is associated with *CIITA* and *RFX5*, two transcription factors that control expression of MHCII[9,17]. In the context of IBD, it remains to be determined how MHCII alleles or perturbations in their expression impact T cell function and pathological inflammation. MHCII variants may qualitatively alter the spectrum of peptides displayed to T cells, thus impacting central tolerance, peripheral tolerance, T cell maintenance, attrition, and/or functional diversification. Interactions

between the intestinal microbiome and host adaptive immune system require early life exposure for establishing tolerance[87]. Defining how commensal epitopes impact T cell development and function, is an emerging new field. The development of new technologies for TCR sequencing and profiling antigen-specific T cells, opens opportunities for specifically studying the unique functional attributes of pathogenic versus protective T cells and tracking the evolution of the TCR repertoire during the course of disease[88,89].

While IBD genetic studies clearly implicate pathways regulating development and function of conventional alpha/beta T cells, emerging evidence indicates that mucosal immunity relies on several unique lymphocyte lineages that recognize conserved microbe-associated ligands presented by nonclassical MHC molecules and/or that respond to cytokines independently of antigen recognition. The cytokine profiles associated with gamma/delta T cells, NKT, and MAIT cells are diverse and overlap with key effectors implicated by IBD genetics such as IFN-γ, IL-17, and IL-13. These cytokines are also produced by specialized innate lymphocyte subsets that lack antigen receptors, such as ILCs, which function as cytokine sensors that rapidly respond to cytokine stimulation by secreting cytokines of their own[90]. For example, local production of IL-23 in the lamina propria by mononuclear phagocytes exposed to commensal bacteria can stimulate ILC3 cells to produce IL-17 and IL-22, which in turn act on epithelial cells to coordinately amplify inflammation and promote healing. In addition to this IL-23 mediated cytokine circuit, IL-1b produced by mononuclear phagocytes can stimulate ILC3 cells to produce IL-2, which functions as a survival and expansion signal for intestinal Tregs[91]. Many additional examples of lymphocyte cytokine networks have been described in the context of intestinal homeostasis and inflammation[92].

Redefining Cytokine Networks.

Efforts to define the cellular heterogeneity of the immune system have revealed an astounding number of cell types that coordinate their unique functions through cytokine-mediated intercellular communication networks[92]. Genes associated with IBD are well represented within these cytokine networks and provide a unique perspective on how genetic vulnerabilities to inflammatory pathology impact cellular crosstalk. Some of the first clues came from genetic associations of *IL10RA* and *IL23R* with IBD[93]. Mechanistic and functional studies have subsequently demonstrated that macrophage-specific deletion of *Il10ra* results in severe colitis in mice[25]. Thus, in the absence of IL10R signaling, macrophages produced elevated levels of IL-23 that induced IL-22 production by conventional T cells. In turn, IL-22 induced intestinal epithelial cells to express chemokines that subsequently recruited neutrophils to the intestine and amplified inflammatory pathology[25] (Figure 3). Counteracting this cytokine loop, Tregs were shown to suppress macrophage IL-23 and IL-1b production, thus limiting inflammation[94]. The IL- 23 cytokine network is particularly well represented in IBD genetics. Exome sequencing identified coding variants in *IL23R* that are associated with protection from IBD onset[20], and GWAS identified the *IL12B* locus as associated with IBD risk[12]. IL-23 is a heterodimer comprised of the protein products of the *IL12B* and *IL23A* genes. IL-23 is primarily produced by mononuclear phagocytes and acts on several target cells including Th17, ILC3, and innate-like lymphocytes. In T cells, IL-23 and IL-6 cooperate to induce the Th17 transcription

factor RORgt[95], a nuclear receptor encoded by *RORC*, a recently identified IBD risk gene. In addition to *RORC*, *IL23R*, and *IL12B*, additional IBD risk genes may function to stabilize or promote Th17 differentiation, such as *IL6ST*, *TYK2*, *GPR65*, and *STAT3* which are all located in IBD risk loci[9]. In fact, patients bearing TYK2 variants that are associated with protection from IBD exhibit impaired T cell responses to IL-23 stimulation and reduced STAT3 phosphorylation[13]. Collectively, several IBD risk genes complement IL-23 stimulation in lymphocytes with a primary effect on promoting production of IL-17 family cytokines and IL-22. These examples illustrate the value of functionally mapping genetic vulnerabilities to cellular communication networks.

Studies of IBD genetics inspired therapeutic approaches aimed at blocking the IL-23/17 axis. Paradoxically, antibodies against IL-12 and/or IL-23 show efficacy in IBD and psoriasis, while inhibition of IL-17 or IL17RA shows efficacy in psoriasis and may exacerbate IBD[8,96]. These observations suggest that IL-17 has important ancillary effects in mucosal tissues related to host defense and barrier function[96]. In mouse models, IL-17F-deficiency was associated with impaired antimicrobial peptide production in the epithelium, leading to a bloom of commensal bacteria (Clostridium cluster XIVa) that promoted Treg development[97]. Thus, IL-17F knock-out mice with elevated Treg frequencies exhibited reduced pathology in models of colitis[97]. Additional studies have provided a different view of IL-17 cytokines demonstrating redundancy in IL-17A and IL-17F for induction of colitis in mouse models[98]. It remains unclear how IL-17R signaling promotes inflammation versus healing in the mucosa, and which cells mediate these differential effects. Functional dissection of genetic risk factors in the IL17RA signaling pathway may reveal the cell types and biological contexts in which the pathway is dysregulated in disease.

In addition to the IL-23/17 axis, IBD genetics highlights specialized cytokine networks that are adapted for immunity at barrier tissues, namely the IL-1 family of cytokines. IL-1b is primarily produced by phagocytes in response to microbe-associated molecular patterns (MAMPs) that induce transcription of IL-1b mRNA, while inflammasomes promote proteolytic activation of IL-1b and assembly of gasdermin pores, facilitating noncanonical secretion of the active cytokine and induction of pyroptosis[99]. In IBD patients with *IL10RA* loss of function variants, excess IL-1b produced by macrophages led to excess inflammation, and treatment with the IL1R antagonist anakinra ameliorated symptoms in subjects that were previously refractory to treatment[100]. IL1R antagonism also reduced IBD symptoms in CGD patients with perianal disease, although it is important to note that these were case studies rather than controlled clinical trials[101]. Additional IBD genetic risk factors impact IL-1b signaling. The CD risk allele *ATG16L1* T300A is associated with elevated IL-1b production in phagocytes stimulated with MAMPs, thus implicating autophagy in lysosomal degradation of inflammasome macromolecular signaling complexes[102–104]. Similarly, ATG16L1 has been suggested to control autophagy-dependent turnover of TRIF oligomers induced by TLR signaling, and the *ATG16L1* T300A allele was shown to augment IFNb and IL- 1b production in response to TLR engagement[105].

Multiple distinct inflammasome complexes regulate IL-1 family cytokines in the context of mucosal immunity and IBD. In addition to IL-1b, inflammasomes control the activation of IL-18, and exome sequencing identified variants in the IL-18 receptor signaling chain

(*IL18RAP*) that are associated with IBD[20]. Moreover, mutations in *NLRC4* are associated with elevated IL-18 levels and severe infantile enterocolitis[30,106]. Additional examples of inflammasome hyperactivation have been described for the Pyrin inflammasome, which is encoded by the *MEFV* gene[107,17] (Figure 3) and *NLRP7*[108].

Intestinal pathologies associated with IL-1 family cytokines extend beyond the inflammasome- dependent cytokines to include family members that function as alarmins in mucosal tissues. Accordingly, IL-33 is an IL-1 family member that is expressed intracellularly in epithelial cells and stroma. Upon physical tissue damage or cellular necrosis, IL-33 is released into the tissue microenvironment where it acts on a diversity of immune cell types expressing its receptor ST2, which is encoded by *IL1RL1*, a gene implicated by IBD fine mapping GWAS[12]. Thus, IL-33 is thought to contribute protective and pathological functions in the mucosa by promoting Treg- mediated tolerance[109], inducing type 2 cytokine production in ILC2 cells[110], and promoting mast cell activation[111]. Similar to IL-33, the IL-36 subfamily of cytokines function as tissue alarmins. IL- 36 family cytokines perform important immune functions at barrier tissues such as skin and gut, thus implicating these cytokines in psoriasis and IBD[92]. Specifically, CD patients with fibrostenotic disease exhibited elevated levels of IL-36 in intestinal biopsies[112]. Inhibition of IL36R by administration of blocking antibody or genetic deletion of the receptor (*Il1rl2*) reduced inflammation in mouse models of epithelial injury[112]. Further functional mapping of these alarmin networks will help identify mechanisms of intestinal homeostasis and provide insights into mechanisms of disease.

Intrinsic Cell Stress Pathways.

Chronic inflammation driven by cytokines and inflammatory mediators in the intestine imposes environmental and metabolic stress on tissues and cells. Early evidence linking IBD to cellular stress pathways was derived from GWAS identifying polymorphisms in autophagy-related genes *IRGM* and *ATG16L1*[113–115]. Autophagy is a cellular disposal system that directs cytoplasmic cargo into lysosomes for proteolytic degradation. Given the central role of autophagy in recycling biomass such as organelles, autophagy integrates cellular metabolism and catabolism to meet the energetic demands of the cell. In addition, autophagy machinery plays diverse roles in several cellular processes including intracellular host defense, lysosome homeostasis, and cellular secretion. Thus, autophagy and lysosome homeostasis intersect with multiple cellular systems including regulators of vesicular trafficking such as the kinase LRRK2. The LRRK2 G2019S coding variant is associated with CD and Parkinson's disease, diseases with different clinical manifestations but surprising genetic overlap[17,116]. *LRRK2* variants may impart IBD risk by impairing antibacterial phagolysosomal disposal in intestinal phagocytes[117] and impart risk of Parkinson's disease by impairing phagolysosomal disposal in microglia or mitophagy in neurons[116]. Indeed, several additional IBD risk genes, including *GPR65*, are associated with lysosome dysfunction and autophagy[34].

Defining the complex roles of autophagy in IBD has been facilitated by the generation of knock- in mice that model the CD risk variant *ATG16L1* T300A. These mice exhibit several cell type- specific phenotypes that are thought to cumulatively contribute to IBD risk in

humans. For example, *Atg16l1* T300A knock-in mice exhibit defective antibacterial autophagy against enteric pathogens[104]. Macrophages derived from T300A mice produce elevated levels of IL-1b in response to inflammasome stimulation[103]. Finally, Paneth and goblet cells derived from *Atg16l1* T300A knock-in mice exhibit granule anomalies associated with impaired secretion of antimicrobial peptides[103] and mucus. Similarly, CD patients bearing *ATG16L1* T300A alleles exhibit abnormal numbers and morphology of Paneth cell granules that segregate CD into distinct pathological subsets[118,119]. Taken together, the *ATG16L1* T300A allele conspires with environmental triggers to induce several cell type-specific phenotypes resulting in tissue dysregulation and predisposition to inflammatory pathology.

The autophagy pathway is intertwined with the integrated stress response. The ATG16L1 T300A substitution creates a caspase 3 cleavage site, rendering ATL16L1 susceptible to caspase cleavage, thus functionally connecting autophagy with apoptosis[103,104]. Cell-extrinsic and -intrinsic stressors prime genetic susceptibility to intestinal pathology in the context of ATG16L1 insufficiency. CD patients bearing *ATG16L1* T300A alleles exhibited pathological ER stress in Paneth cells, which was specific to the T300A genotype and was even evident in quiescent disease[120]. ER stress responses control epithelial barrier function, thus coordinating interactions of the adaptive immune system with luminal antigens. Healthy subjects bearing *ATG16L1* T300A alleles exhibit signs of elevated epithelial ER stress and ensuing polyreactive IgA responses[121]. Thus, *ATG16L1* T300A functions to integrate autophagy and ER stress pathways in the intestinal mucosa.

The ER stress pathway plays a central role in epithelial barrier function. Conditional deletion of key ER stress sensors and effectors in the intestinal epithelium results in pathological, unresolved ER stress and spontaneous enterocolitis[122]. Thus, secretory epithelial cells such as Paneth cells and goblet cells require highly efficient ER secretory machinery to maintain barrier integrity and innate immunity[123,124]. Genetic vulnerability in cell stress pathways can predispose secretory epithelial cells to developing pathological ER stress culminating in apoptosis, barrier breach, and chronic inflammation. Recent studies have identified genetic risk factors associated with IBD that act through regulation of ER stress, such as *TMEM258*, which is located in a locus associated with CD[9]. Mechanistically, TMEM258 functions as an essential subunit of the oligosaccharyltransferase complex, which controls N-linked protein glycosylation in the ER and is essential for directing export of nascent proteins through the secretory pathway[125]. Several additional IBD risk genes have been associated with defective protein glycosylation defects and congenital disorders of glycosylation such as *SLC39A8*[126] and *RFT1*[127] (Figure 3). These findings demonstrate the important role for protein glycosylation in facilitating protein trafficking through the secretory pathway, thus linking ER stress to epithelial function in the intestine.

Metabolism.

The inflamed intestinal mucosa in IBD is paradoxically associated with elevated oxidative stress and signs of hypoxia[37,128]. Inflammation can rapidly disrupt oxygen gradients in the mucosa and lead to fluctuations that perturb homeostasis and exacerbate tissue damage. Innate immune responses are highly oxygen-consumptive and generate toxic free radicals.

Thus, host cells and tissues adapt to oxidative stress and hypoxia in a coordinated manner by engaging two interrelated transcriptional responses controlled by NRF2 and HIF-1a respectively.

While superoxide and reactive oxygen intermediates (ROI) are potent antimicrobicidal agents, they induce oxidative stress, and their production consumes local oxygen leading to hypoxia and HIF-1a activation[129]. In turn, HIF-1a induces a metabolic shift towards glycolysis and initiates angiogenesis resulting in a rebound in tissue oxygenation that can help reestablish normoxia and healing or exacerbate oxidative stress. HIF-1a activation by means of an inhibitor of CUL2 neddylation, which stabilizes HIF-1a, enhances epithelial barrier integrity and reduces pathology in models of epithelial injury[130]. IBD GWAS identified *CUL2* as a risk gene, and exome sequencing identified a rare variant that disrupts mRNA splicing that is associated with protection from IBD[20]. In contrast to HIF-1a, transgenic overexpression of HIF-2a exacerbated pathology associated with epithelial injury[131,132]. HIF-2a uniquely controls expression of genes in the creatine metabolism pathway, including creatine kinases and the creatine transporter SLC6A8, which collectively regulate the phosphocreatine shuttle to provide energy required for remodeling of epithelial junctional complexes[133]. Together, HIF-1a and HIF-2a control cellular metabolism and adaptation to environmental demands imposed by inflammation, suggesting that rebalancing HIF- 1a and HIF-2a locally may be a viable therapeutic strategy.

Leukocytes must rapidly switch from quiescent to activated states to elicit effector function in a controlled manner. This requirement necessitates metabolic versatility to fuel effector responses and conserve energy during quiescence. In macrophages, TLR engagement leads to a shift from oxidative phosphorylation to glycolysis, resulting in accumulation of the tricarboxylic acid cycle (TCA) intermediate succinate, which promotes stabilization of HIF-1a to enhance transcription of IL-1b[134] and also regulates type 2 immunity through its effects on tuft cells[135]. Another TCA intermediate, aconitate, is converted to itaconate by Irg1, which is an enzyme that is induced as part of the interferon response[136]. Itaconate is an electrophilic compound that alkylates key cysteine residues in Keap1, thus relieving inhibition of Nrf2, which elicits the antioxidant response and downregulates inflammatory cytokine production[137]. Thus, immunometabolism is intimately linked with inflammatory and cell stress pathways associated with IBD. In this context, the enigmatic enzyme LACC1 (C13orf31) was identified in genetic studies as associated with CD, leprosy, and other inflammatory diseases[9]. Lacc1 knock-out mice were shown to be highly susceptible to severe histopathology in response to the enteric pathogen *Citrobacter rodentium* or collagen-induced arthritis (CIA)[138]. In these models, elevated levels of IL-17A were observed in Lacc1-deficient mice, although it remains unclear if this phenotype is a primary or secondary effect of Lacc1-deficiency. Given expression patterns of Lacc1, it is not likely to be a T cell intrinsic phenotype[138]. Instead, *LACC1* is highly expressed in myeloid cells and is thought to regulate immunometabolism to promote antibacterial responses and cytokine production[139,140]. The CD risk variant LACC1 I254V was shown to exhibit impaired function associated with defective antibacterial responses[139,140]. Although the specific metabolic function of LACC1 is incompletely understood, it has been proposed to regulate mitochondrial respiration and/or fatty acid oxidation[140]. Many more metabolic processes

and intermediates are likely to be discovered and attributed with immunomodulatory properties that impact IBD.

Tissue Stroma, Inflammation, and Fibrosis.

Inflammation and metabolic stress regulate tissue homeostasis and immunity at barrier sites[112,141]. In this context, chronic inflammation associated with autoimmune diseases frequently elicits a pathological inflammation-healing cycle leading to tissue damage from fibrosis and scarring. This core pathological pathway is conserved across disparate organ-specific autoimmune diseases but has distinctly tissue-specific effects in IBD. Although significant progress has been made in targeting inflammatory pathways in IBD, a significant proportion of patients are refractory to these treatments and develop severe complications associated with fibrosis, which ultimately require surgical intervention. Thus, there is an unmet need for therapeutic strategies aimed at preventing the onset of fibrosis, suggesting that we are missing key target pathways underlying fibrosis.

Several clinical studies have examined treatment-refractory patients and derived gene expression signatures from intestinal biopsies to define the nonresponsive state[5,48]. While these studies examining gene expression signatures in bulk tissue identified a clear signature associated with treatment resistance, they do not directly reveal the cellular source(s) of this pathogenic response. By overlaying the treatment-resistance signature onto a single cell transcriptomic map of UC, fibroblasts were shown to be the dominant cell type of origin for this signature[45]. Specifically, the anti-TNF-resistance signature was enriched in inflammation associated fibroblasts (IAFs) and included genes such as *IL13RA2*, *TNFRSF11B*, and *IL11*[45]. These findings corroborate previous studies delineating a cellular signaling circuit that is actively engaged in the TNF-resistant state, wherein IAFs are activated by the IL-6 family cytokine OSM to produce chemokines that recruit neutrophils to the inflamed mucosa[48] (Figure 3). Thus, inflammatory myeloid cells communicate with fibroblasts through OSM, which signals through a heterodimeric receptor encoded by *OSMR* and *IL6ST*[48], two genes implicated in IBD GWAS.

Based on the link between *IL6ST* and IBD, and the fact that IL6ST (gp130) functions as a common signaling receptor for multiple IL-6 family members, the pathological effects of *IL6ST* variants may span multiple cytokine networks and cellular functions. For example, *IL6ST* and *IL11RA* together encode the receptor for IL-11, which is highly expressed in IAFs and associated with the anti- TNF-resistance signature[45]. IL-11 is induced in fibroblasts in response to stimulation with TGF-b, and subsequently engages an autocrine loop in fibroblasts resulting in deposition of extracellular matrix[142]. Inhibition of IL-11 signaling ameliorates cardiovascular fibrosis in mouse models[142], suggesting that IL-11 may control the arm of the TGF-b response that drives fibrosis. Indeed, TGF-b exhibits pleiotropic effects on tissue development and extracellular matrix remodeling. This is exemplified by the complex phenotypes associated with *TGFB1*-deficiency in humans, which include severe IBD and encephalopathy[143]. Thus, targeting fibrosis by blocking TGF-b may result in adverse effects, but identifying alternative targets controlling defined pathways in fibrosis may offer viable alternatives. For example, IL-11 and IGFBP3 are highly enriched in intestinal fibroblasts derived from UC patients[44,45] and have been

previously implicated as profibrogenic factors[142,144]. Moreover, *IGFBP1–3* were identified as genetic risk factors for severe complications associated with CD prognosis in GWAS[86]. Taken together, evidence from genetics and clinical studies continue to provide clues into the contribution of fibrosis pathways in IBD pathogenesis.

Therapeutics

Influence of Genetics on IBD Therapies.

IBD genetic studies have been instrumental in identifying pathways associated with disease risk and that function as mechanistic drivers of disease pathology. Thus, insights from genetics have direct implications for classifying IBD into clinical subsets based on underlying molecular pathomechanisms and devising more focused therapeutic interventions. The current standard of care for CD and UC is similar, despite recognized differences in clinical and molecular features. Corticosteroids, aminosalicylates, occasionally thiopurines and antibiotics remain the first line treatment for mild to moderate CD and UC. Treatment of moderate to severe cases of IBD has benefited from the recent development of biologics targeting cytokines (TNF or IL-12/23) or leukocyte trafficking receptors (alpha4 beta7 integrin). Historically, therapeutic strategies deployed for IBD have focussed on treating inflammation. Decades of clinical practice have led to the optimization of treatment regimens, although it is not entirely clear how and when these approaches will succeed or fail to achieve efficacy in individual patients. New insights are emerging from IBD genetics that show great potential for illuminating the mechanisms of disease, defining mechanisms of action for treatment interventions, and nominating new candidate targets for therapeutic development.

The majority of therapeutics that are currently utilized to treat IBD were developed prior to the maturation of IBD genetics. We anticipate that genetics and functional genomics will continue to play an important role in advancing the development of novel therapeutic strategies. With that said, emerging evidence from IBD genetics provides rationale that helps explain the efficacy of therapeutics that were developed many years ago. For example, anti-TNF has been a mainstay for the treatment of IBD and several autoimmune diseases. While it is still not entirely clear how TNF inhibition leads to remission in IBD, there are clues from genetics that TNF shedding mediated by ADAM17 proteases[17,145] and TNFR signaling through RIPK1[146] are important contributors to pathogenesis. These observations provide insights into how anti-TNF may function in patients, what pharmacodynamic markers might help in evaluating efficacy, and how alternative targets in these pathways may be exploited.

Some of the newer biologics used to treat IBD, can be rationalized by genetics. The most recent IBD GWAS implicated a number of novel risk genes encoding integrin subunits[11]. This finding supports clinical observations that inhibition of leukocyte trafficking through integrin blockade (anti-alpha4beta7 antibodies) or antagonists of S1PR exhibit efficacy in IBD[147,148]. Additionally, protective variants in *IL23R* predict the observed efficacy of anti-IL-23 in IBD patients[20,21,93]. Although IL-23 is a major driver of IL-17 production by T cells and ILCs, biologics targeting IL-17 or IL17RA have not demonstrated convincing efficacy in IBD[96]. Thus, IL-17 appears to have pleiotropic effects on mucosal homeostasis,

some of which are beneficial, and clues from IBD genetics may help dissect the differential mechanisms of IL-17 signaling in target cells. Perhaps emerging insights from genetics will help pinpoint optimal points of entry within complex cytokine pathways. For example, fine mapping IBD GWAS loci implicated *JAK2* as a causal risk gene[12], thus suggesting that targeting multiple inflammatory cytokine receptor pathways through JAK inhibition may be a viable therapeutic strategy. Indeed, pan-JAK inhibitors have shown efficacy in clinical trials for IBD[147,148].

Taken together, IBD genetics has identified genetic vulnerabilities within integrated pathway networks that regulate homeostasis between the immune system, mucosal barrier tissues, and the microbiome. These genetic vulnerabilities represent key regulatory nodes in the network that may be “tunable” in response to targeted therapeutic interventions that aim to restore mucosal homeostasis. Here, the challenging therapeutic objective is to restore intestinal homeostasis in individuals, regardless of the genetic and environmental perturbations that may have initially triggered network dyshomeostasis. Recent insights from IBD genetics and functional genomics suggest novel strategies for targeting genetic vulnerabilities and mimicking protective variants.

Outlook

The Future.

The field of IBD genetics has advanced quickly in the genomics era. Ongoing efforts have made great strides in synthesizing human genetics with functional genomics, patient-based research, animal models, and mechanistic studies. Accordingly, the genetic architecture of IBD has been elaborated to provide functional context by mapping genes-to-pathways. Deeper insights into disease pathogenesis are emerging from exome sequencing and subsequent experimental conversion of variants-to-mechanisms. Collectively, these efforts offer new directions for solving outstanding problems in clinical management of IBD, and more broadly, for shedding light on mechanisms of immunoregulation. Indeed, IBD can be viewed as a model disease to dissect mechanisms of mucosal immunity; however, IBD is a complex phenotype that is impacted by multiple pathways. Parsing and condensing common variants into functionally related polygenic pathway scores may help reclassify patients based on molecular phenotypes. Clinical studies examining cohorts defined by pathway risk scores, and individuals at the extreme ends of the risk spectrum, may facilitate biomarker discovery and identify disease mechanisms that inform targeted therapeutic interventions. Moreover, developing high fidelity biomarkers and clinical assays to quantify key pathways in IBD will be instrumental in redefining genotype- phenotype relationships. These efforts are catalyzed by public databases such as the UK Biobank and FIMM, which include large cohorts of individuals that have undergone exome sequencing and incorporate extensive clinical metrics with genotype data. These biobanks can be powerful engines for discovery, especially if they grow to incorporate informative clinical metrics of IBD- relevant pathways, such as intestinal permeability, immune function, and serum titers for common vaccines, to name just a few.

Much of the work to date in human genetics has focused on heritable genetic variants, while studying somatic variants may offer another opportunity to identify mutations in pathways

that impact pathology. For example, biopsies from inflamed regions of the colon may contain more and qualitatively different somatic mutations compared to non-inflamed regions. In this regard, inflammation may positively select for somatic mutations in signaling pathways that render epithelial and immune cells resistant to cytokine toxicity. While it remains to be determined if somatic mutations associated with inflammation contribute to the pathogenesis of IBD, there is a clear precedent for inflammation inducing somatic mutations that drive carcinogenesis. Future studies aimed at finding genes that are under positive selection pressure in the context of intestinal inflammation may help identify key pathways associated with IBD pathology.

Genetics has provided unique insights into functional pathways that mediate homeostasis between the immune system and mucosal tissues. Recent research has shed light on how the microbiome interacts with host genetics to modulate intestinal homeostasis[84]. There are clear associations between host genetic variables and features of microbial dysbiosis in the microbiome that are downstream consequences of perturbed immune function. The challenge moving forward will be to identify compelling statistical associations between host genetics and metagenomic pathways that reveal direct mechanistic interactions between microbes and the host. Integrating host and microbiome genetics remains an area of active investigation[3], which has benefited from years of research functionally annotating the human genome. In this context, functional gene annotations are subject to revision over time as experimental evidence accumulates. Thus, there is a need to accelerate the generation of high-quality empirical data such as protein-protein interaction networks derived from relevant cell types and functional genetic screens in the context of key IBD pathways. Defining more accurate gene-pathway annotations, will significantly accelerate the process of assigning function to disease-associated variants. It is important to note that the genetic variants associated with human disease may exhibit similar or different phenotypes compared to null alleles. While genetic knockout studies are invaluable for assigning gene function, it is essential to study the mechanisms of action for variants associated with human disease.

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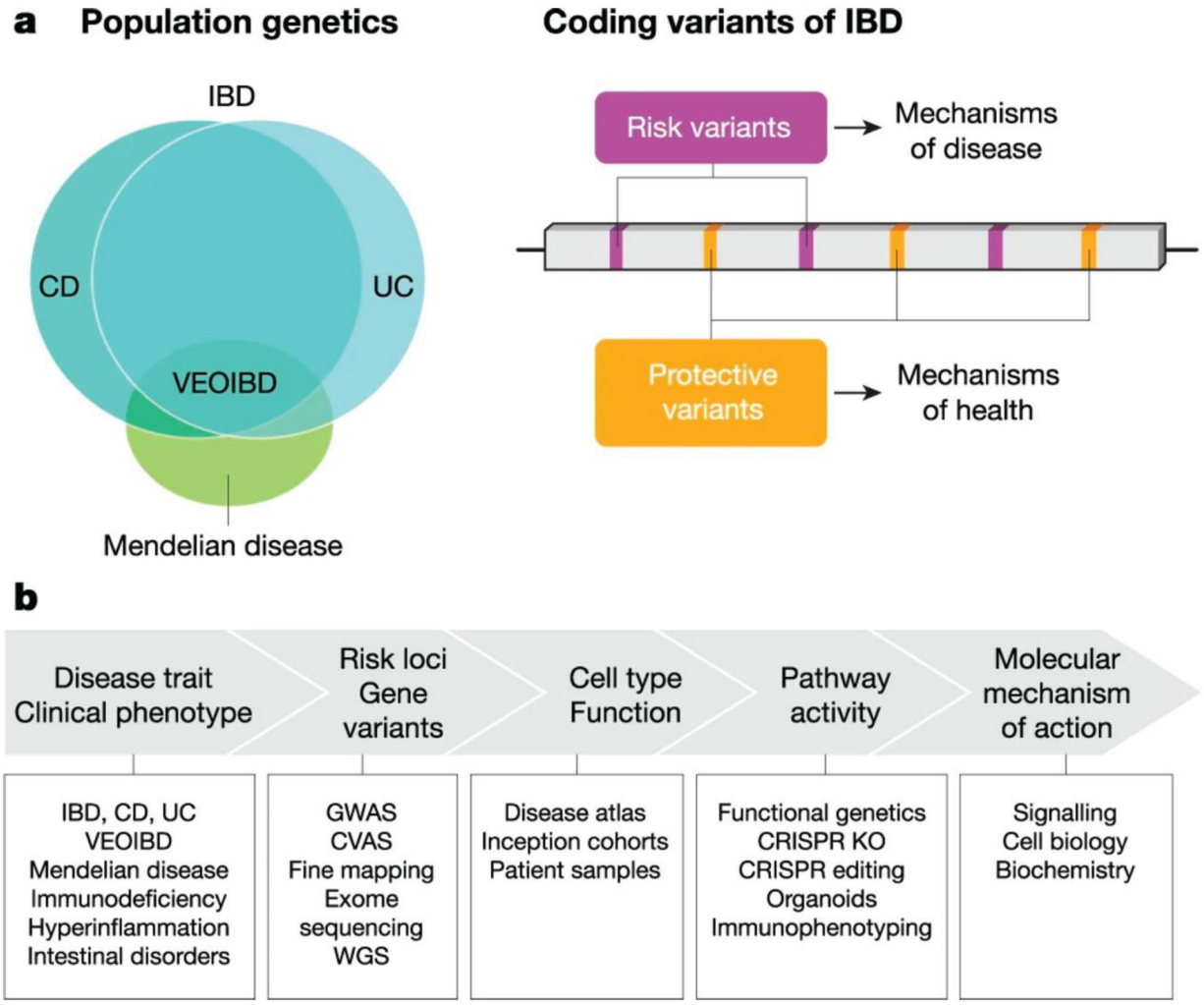

Figure 1. Strategies in human genetics and functional genomics to dissect mechanisms of disease.

a, Left, IBD is a complex genetic disease that is affected by many genetic risk factors defined in common variant association studies (CVAS). These genetic risk factors partially distinguish disease phenotypes associated with CD versus UC. Rare genetic variants associated with severe IBD and very early-onset (VEO)IBD exhibit Mendelian inheritance patterns and have helped to identify genes that control intestinal homeostasis. Similar insights have been gained from rare genetic variants associated with primary immunodeficiencies and hyperinflammatory disorders that manifest with intestinal pathologies or those that confer protection from these conditions. Right, mechanistic study of individual coding variants comprising an allelic series for a gene can reveal molecular functions at the protein level. Understanding the molecular function of a risk variant can reveal mechanisms of disease and conversely, study of protective variants can reveal mechanisms of health. **b**, Functional genetics has helped to mechanistically link risk genes and genetic variants with cellular and molecular functions underlying the disease process. KO, knockout; MOA, mechanism of action; WGS, whole genome sequencing.

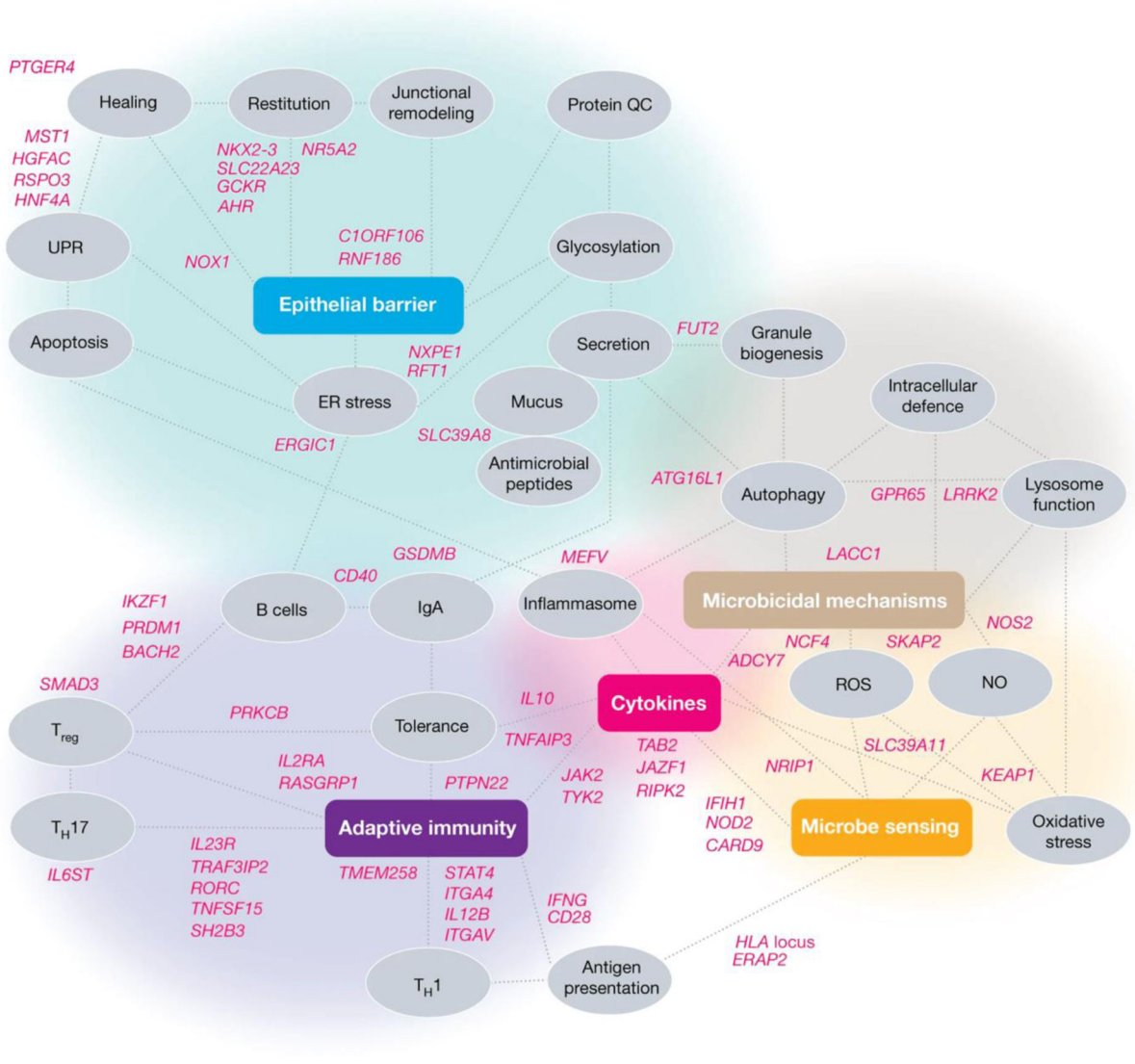

Figure 2. IBD genes and pathways controlling mucosal immunity.

IBD risk genes regulate a complex network of interconnected functional pathways. IBD genes (red text) have been implicated in key biological functions (grey circles) that are controlled by interconnected molecular pathways (coloured rectangles). Lines connecting nodes reflect overlapping molecular regulation by common genes. Several IBD risk genes regulate several distinct biological functions depending on their cell type-specific activities.

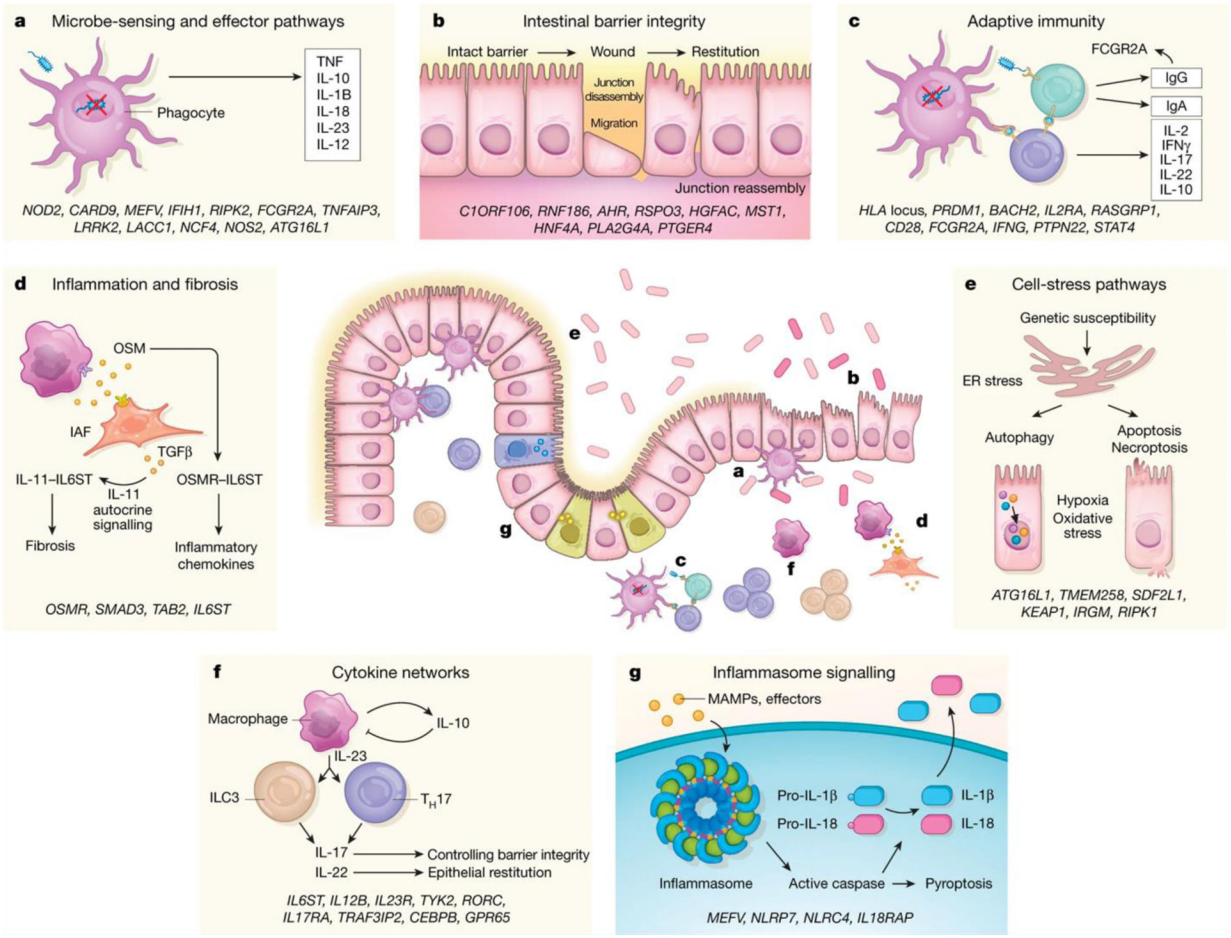

Figure 3. Pathway paradigms highlighted by IBD genetics.

IBD risk genes listed at the bottom of each panel represent genetic vulnerabilities that perturb key pathways underlying intestinal homeostasis and drive inflammatory pathology in the gut mucosa (centre). **a**, Phagocytes have evolved numerous mechanisms to detect microorganisms and elicit effector responses that promote inflammation through cytokines and antimicrobicidal responses such as oxidative burst and xenophagy. **b**, Intestinal epithelial cells maintain barrier integrity through dynamic remodelling of junctional complexes. **c**, Coordinated interactions between dendritic cells, T cells and B cells facilitate the induction of antigen-specific immune responses and immunological memory directed against commensal microorganisms. **d**, Stromal cells—such as fibroblasts—are key mediators of tissue remodelling and healing that dynamically respond to inflammatory conditions. **e**, Cell-extrinsic and -intrinsic stressors associated with inflammation sensitize cells to death. The integrated stress response facilitates adaptation to these stressors by coordinating cellular responses to ER stress, oxidative stress, hypoxia, autophagy and cell death pathways. **f**, Intercellular communication among immune cells, stroma and epithelial cells is tightly controlled by elaborate cytokine networks. **g**, Microbe-sensing pathways that operate in the cytosol of host cells function to detect and respond to intracellular infection or exposure to bacterial toxins.

Distinct inflammasome complexes mediate activation and secretion of IL-1β or IL-18 and elicit pyroptosis, a proinflammatory form of programmed cell death.

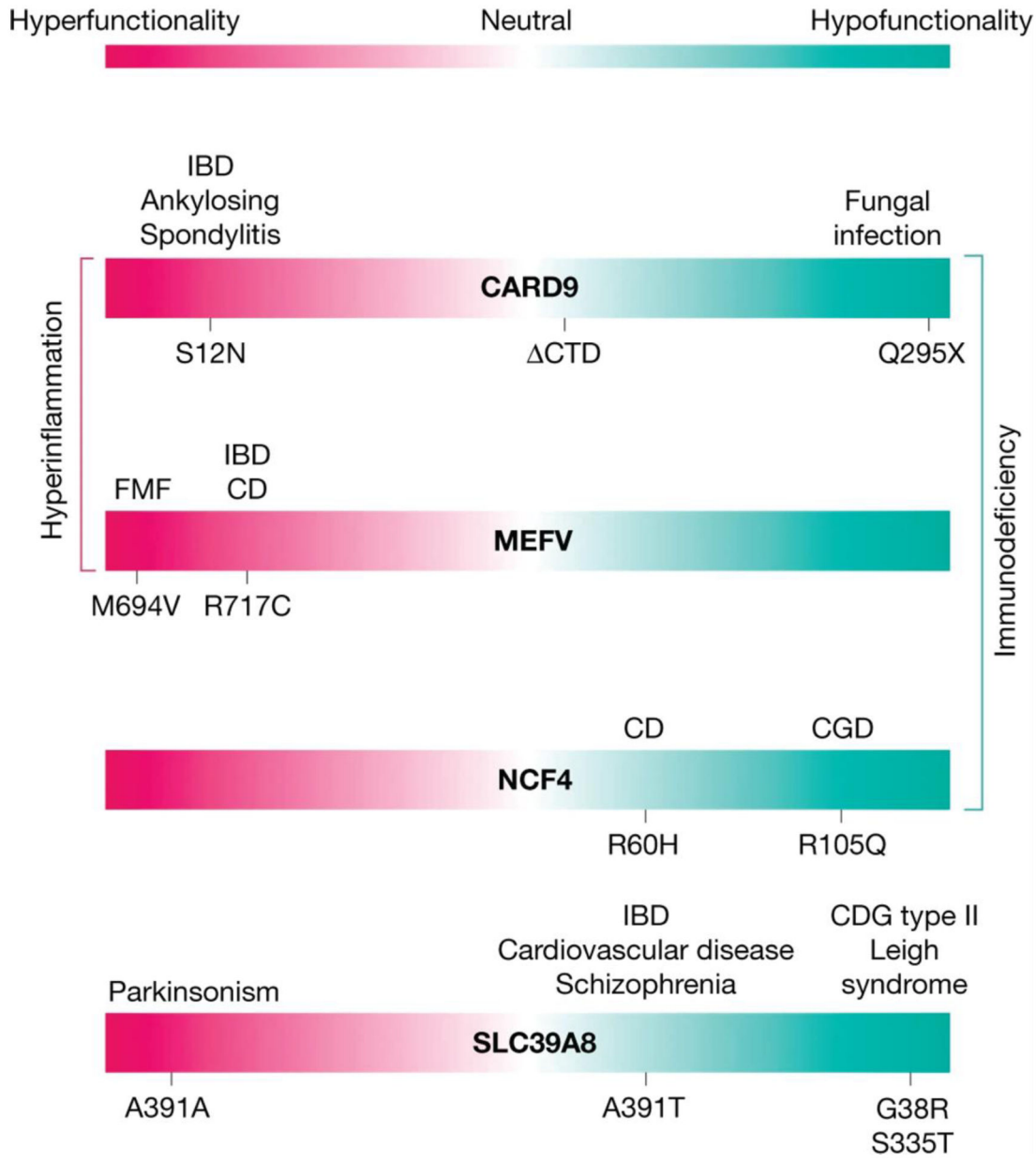

Figure 4.

Exome sequencing has identified many IBD risk genes (middle) with allelic series associated with a spectrum of phenotypes (left to right). Top, for example, *CARD9*^{S12N} is associated with risk of IBD, whereas *CARD9*$^{\Delta CTD}$ is associated with protection and *CARD9*^{Q295X} is associated with an immunodeficiency linked with chronic life-threatening fungal infections. Other IBD genes contain coding variants with distinct phenotypes and disease associations that may offer clues to understanding mechanisms of IBD pathology. FMF, familial Mediterranean fever; CGD, chronic granulomatous disease; CVD, cardiovascular disease; CDG, congenital disorder of glycosylation.