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Insights into IBD Pathogenesis

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Abstract

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disorder caused by dysregulated immune responses in a genetically predisposed individual. Recent accumulating data, including genome-wide association studies, have identified more than 50 distinct genetic loci that confer susceptibility. We highlight the role of microbial-host interaction, particularly with respect to the overlap of common genetic and pathophysiologic mechanisms of CD and UC, interleukin-22-producing natural killer cells, autophagy, and TL1A, a member of the tumor necrosis factor (TNF) family, in gut homeostasis and IBD pathogenesis. This article focuses on the recent advances in understanding of IBD from the past year, including advances in genetics and immunobiology.

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are two disparate forms of inflammatory bowel disease (IBD) that share related characteristics, but have unique clinical distinguishing features. Genome-wide association studies (GWAS) have identified more than 50 genetic associations with CD and UC. Similar to clinical phenotypes, UC and CD share genetic susceptibility loci but differ at others, suggesting common genetic and pathophysiologic mechanisms that underlie these two disease entities. In addition to the evidence indicating that CD occurs when commensal, opportunistic, or pathogenic bacteria initiate and perpetuate a dysregulated mucosal immune response, recent UC GWAS also revealed genetic association between UC and genes important in host defense against infection, highlighting the importance of bacterial-host interaction in IBD.

The main genetic associations in IBD can be divided into genes that contribute to the innate and adaptive immune response. In the innate immune arm, the association of CD with polymorphisms in *NOD2* (*CARD15*) and the two autophagy-related genes, *ATG16L1* and *IRGM*, implicate defects in the recognition and handling of intracellular bacteria in the immunopathogenesis of IBD. Recent studies have shown that human and mouse gut-associated lymphoid tissues harbor a unique natural killer (NK) cell subset that produce interleukin (IL)-22, a cytokine that plays a role in host defense and autoimmune diseases, including IBD. In the adaptive immune arm, CD has been considered a condition driven by T-helper 1 (Th1) and/or Th17 whereas UC appears to be Th2 dominant [1]. However, recent genetic association studies indicate that overlapping immunogenetic pathways, especially the Th17 inflammatory axis, drive UC. Tregs, an immune-modulating subset of CD4⁺ T-cells, can suppress the differentiation and function of Th1 and Th2 cells. The immunopathologic concept of IBD is evolving in light of recent studies that unveiled novel effector pathways in IBD, one of which

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is the TL1A-DR3 pathway in microbial-host interaction and bridging innate and adaptive immune response.

GWAS and Overlapping Immunogenetic Pathways Between IBD Subtypes

GWAS examine tens to hundreds of thousands of single nucleotide polymorphisms (SNPs) across the human genome in cases and controls. Simply, the allele frequencies of these SNPs are statistically compared between cases and controls to determine any association between the SNP and the disease or condition in question. Independent confirmation of association is often performed for findings generated in a GWAS. Therefore, any confirmed SNP associations that are seen with a GWAS are likely to be in linkage disequilibrium with a true disease susceptibility allele. Several GWASs have been performed in IBD (Table 1) [2,3•,4–12].

Although CD and UC differ in disease localization, endoscopic appearance, histology, and clinical course, common clinical and pathologic features are shared by these two conditions, suggesting that CD and UC share some genetic susceptibility loci but differ at others. Indeed, two recent systematic GWAS of UC revealed overlap of genetic markers between UC and CD including *IL23R* and *JAK2* whereas other markers are specific to UC including *ARPC2*, *PLA2G2E*, and *IL-22* [10,12]. Furthermore, a candidate gene experiment tagging 29 of the CD loci and testing for potential overlap with UC confirmed the overlap between the IBD subtypes [13]. This study also shows an association of *JAK2* and *STAT3* in UC [13]. Some of the downstream targets of the JAK-STAT pathway are the IL12/23 axis, IL23 signaling, and maturation of naïve CD4+ T cells to Th17 cells. Together, the two UC GWAS and the candidate association studies reveal a common immunogenetic pathway between CD and UC, particularly the Th17 inflammatory axis.

Similar to the CD GWAS, the two recent UC GWAS reveal genetic associations that underscore the importance of bacterial-host interaction in IBD. For example, *PLA2G2E* gene is in close proximity to the UC susceptibility SNP rs6426833 [10]. Evidence for *PLA2G2E* in bacteria-mediated inflammation lies in the fact that this family of secretory phospholipase A2 leads to the production of proinflammatory lipid mediators (eg, prostaglandins and leukotrienes) and that bacterial lipopolysaccharide can upregulate *PLA2G2E* expression [14]. Another genetic UC association that implicates the importance of bacterial-host interaction is at SNP rs1558744 in close proximity to *IL-22* [10]. *IL-22* is a member of the family of IL-10–related cytokines, which mediate host defense against infections as well as tissue inflammation in many chronic, immune-mediated disorders, including IBD [15].

Remarkably, despite the discovery of new genetic susceptibility loci with GWAS, the identified IBD markers account for only 20% of the heritable risk [3•]. Thus, a considerable amount of genetic research remains to be done to identify the risk and protective IBD genes to better elucidate IBD pathogenesis.

Innate Immunity

Antigen presentation to T cells by professional antigen-presenting cells (APCs) such as dendritic cells (DCs) is critical for the activation of adaptive immune responses. There are three main pathways to transport luminal antigen to lamina propria (LP). The first is mediated by microfold (M) cells through the capture of luminal antigens and their presentation to T cells. The second pathway, performed by LP DCs, involves dendritic cell extension to the intestinal lumen across epithelial cells, facilitating the capture of luminal antigens (Fig. 1). The third pathway involves the neonatal Fc receptor (FcRn), which serves as the vehicle for transporting LP immunoglobulin G (IgG) across the colonic epithelial layer into the lumen where the IgG can bind enteric bacterial antigens to form immune complexes (IC). The FcRn then recycles

the antigens/IgG IC back across the colonic epithelial cells into the LP for processing by APCs such as DCs, which are capable of presenting the antigens to T cells.

One of the ways that host discerns foreign from self antigen is through pattern recognition receptor (PRR), which recognizes specific molecular patterns of pathogens. One group of PRRs consists of the Toll-like receptors (TLRs) with variable specificities for sensing microbial products. TLR activation can stimulate signaling cascades leading to proinflammatory cytokine production and induction of co-stimulatory signals to further augment innate and initiate effector adaptive immune responses (Fig. 1). One of the mechanisms by which sentinel APCs augment innate immune response is enhancement of the lytic potential of NK cells and their ability to produce interferon (IFN)- γ [15]. Recently, a unique NK cell subset that specializes in the production of IL-22 and is responsive to IL-23 was identified [16]. Several murine studies in the past year point to a protective role for these IL-22-producing NK cells in host defense and IBD [15]. Lastly, genetic associations between autophagy genes and CD highlight the importance of intracellular processing of bacterial components in mucosal homeostasis.

IL-22–Producing NK Cells: Protective Role in Host Defense and IBD

The recent identification of *IL-22* as a UC susceptibility gene implicates this cytokine in the pathogenesis of IBD [10]. IL-22 is a member of IL-10–related cytokines and plays a role in maintaining the integrity of epithelial cell barrier in the gut from pathogens [15]. Binding of IL-22 to its receptor initiates a signal transducer and activator of transcription (STAT) 3 signaling pathway that induces the production of antimicrobial proteins including β -defensin-2, β -defensin-3, S100A7, S100A8, S100A9, RegIII β , RegIII γ , and lipocalin-2, which protects the epithelial barrier against microbes [17,18]. Direct induction of antimicrobial proteins may be one of the pathways for IL-22 in regulating early defense mechanisms against extracellular bacterial infection caused by *Citrobacter rodentium* [18].

Several studies have identified a distinct population of NKp46⁺ cells that express and depend on the ROR γ t transcription factor for their development [16,19,20]. Interestingly, the differentiation of IL-22–producing ROR γ t⁺NKp46⁺ cells is dependent on the presence of gut microflora since germ-free mice have fewer IL-22–producing NK cells [19,21]. Additionally, IL-22 is produced in an IL-23–dependent way during inflammation and promoted the expression of antimicrobial proteins [16,19]. Although IL-23 is important in the differentiation of Th17 cells that produce IL-22 [22,23], several recent reports also demonstrated that IL-23 is an effective trigger of IL-22 secretion in ROR γ t⁺NKp46⁺ NK cells (Fig. 1) [23]. As discussed above, GWAS have identified *IL-23* polymorphisms that are associated with CD and UC. It is noteworthy that IL-22 was found in the two recent GWAS to be associated with UC, suggesting that the IL-23–IL-22 axis is involved in the pathogenesis of UC.

Several recent studies implicated a protective role for IL-22–producing NK cells in IBD. Adoptively transferring CD4⁺CD45RB^{hi} T cells from wild-type (WT) or IL-22–deficient mice into Rag1^{−/−} or Rag1^{−/−}IL22^{−/−} mice showed that the absence of exogenous and endogenous non-T non-B cell sources of IL-22 led to worsened murine colitis than with lack of either exogenous T-cell or endogenous sources of IL-22 [24]. NK cells were identified as the likely population that produced IL-22, the absence of which led to worsened murine colitis [24]. The protective role of IL-22 in mucosal inflammation further demonstrated that IL-22 protected against intestinal *C. rodentium* infection in Rag2^{−/−} mice [18]. Because Rag mice lack T-cell sources of IL-22, the protective role of IL-22 in this model may be from NK cells. Furthermore, using anti-NK1.1 antibody to deplete NK cells resulted in heightened susceptibility to of this type of infection [16,21]. These results implicate that ROR γ t⁺NKp46⁺ NK cells provide an innate source of IL-22 that has a protective role in host defense and mucosal inflammation (Fig. 1).

Defects in Autophagy Associated With Crohn's Disease

Autophagy (from the Greek “auto,” oneself and “phagy,” to eat) is a biologic process of membrane trafficking in which autophagosomes engulf organelles and cytosolic macromolecules, followed by fusion of the autophagosome with a lysosome to form an autolysosome in which sequestered material is degraded. The degraded content can then be loaded onto human leukocyte antigen (HLA) class II molecules or to compartments where recognition by TLR may occur. Autophagy is important for cellular homeostatic functions including structural remodeling, nutrient and energy generation, degradation of damaged or long-lived cytoplasmic components, and protection against invading microorganisms. Its protective role in infectious disease is an important part of the innate immune armamentarium and links to adaptive immunity by delivery of foreign antigens necessary for immune recognition. Several GWAS identified *ATG16L1* and *IRGM* as new CD susceptibility genes [3•,25–27].

The *ATG16L1* gene product is comprised of an N-terminal ATG16 domain thought to be essential for interaction with other autophagy proteins such as ATG5 and ATG12; a coiled-coil domains postulated to mediate homodimeric interactions; and seven C-terminal WD repeats thought to create stable platforms that can coordinate the formation of multimeric protein complex, which is essential for the formation of the autophagosome [1]. *ATG16L1* is broadly expressed by intestinal epithelial cells, APCs, CD4⁺, CD8⁺, and CD19⁺ primary human T cells [1].

The role of *ATG16L1* in mucosal inflammation is being studied in mice with targeted genetic manipulations. Recent data using mice hypomorphic for *ATG16L1* showed that the defect appears to be confined to gut paneth cells [28]. The investigators showed that *ATG16L1* hypomorphic paneth cells exhibited abnormalities in the granule exocytosis pathway characterized by defective antimicrobial peptide secretion [28]. Importantly, similar reduction in secreted antimicrobial peptides was observed in biopsies from a CD patient homozygous for the *ATG16L1* risk allele [28]. This suggests that the human *ATG16L1* risk allele has reduced autophagy function, leading to reduced secretion of antimicrobial peptides by paneth cells.

In another study, *ATG16L1* deficiency in mice macrophages produced high amounts of inflammatory cytokines IL-1 β and IL-18 when these macrophages were exposed to bacterial lipopolysaccharide [29]. The increased production of IL-1 β and IL-18 is due to Toll/IL-1 receptor domain-containing adaptor inducing IFN- β (TRIF)-dependent activation of caspase-1; where caspase 1 is thought to cleave pro-IL-1 β to yield active cytokine [29]. Chimeric *ATG16L1*-deficient mice are highly susceptible to dextran sulphate sodium-induced (DSS) acute colitis, which can be rescued by injection of neutralizing antibodies to IL-1 β and IL-18 [29]. These findings indicate a role of autophagy in regulating the production of proinflammatory cytokines and in controlling bacterial endotoxin-mediated gut inflammation.

Given the important role of autophagy in the control of microbial-induced inflammatory immune response, mutations affecting important factors involved in autophagy may result in immune dysregulation seen in IBD. The association between CD and a nonsynonymous amino acid change—a threonine to alanine substitution at position 300 (*T300A*)—is found in several GWAS [2,7,25]. The role of the *T300A* risk-associated variant was studied in a model of intracellular pathogen infection using *Salmonella enterica serovar typhimurium* [30]. The investigators reported that the *T300A* substitution resulted in a variant of *ATG16L1* that is less stable and has reduced capability to mediate antibacterial autophagy [30].

IRGM is a member of a family of genes encoding interferon-inducible guanosine triphosphatases that are involved in pathogen clearance. *IRGM* is expressed in several tissues including colon, small intestine, peripheral blood leukocytes, and monocytes that have been

found to have an important role in eliminating the intracellular pathogens *Toxoplasma gondii* and *Listeria monocytogenes* [26]. Furthermore, in gene-knockdown experiments, *IRGM* has been shown to induce autophagy to control the intracellular mycobacterial [31] and *Salmonella typhimurium* load [32].

Although multiple *IRGM* SNPs were found to be associated with CD [2], sequencing of the *IRGM* coding region did not detect any causal amino acid-changing SNPs [26]. A recent report found a 20-kb deletion polymorphism upstream of the *IRGM* gene to be strongly associated with CD-associated SNP [32]. Therefore, the genetic variants conferring susceptibility to CD may lie in regulatory sequences that affect either *IRGM* expression, transcript splicing, or the rate of protein translation. Taken together, the associations of SNPs in *ATG16L1* and *IRGM* with CD indicate that proteins involved in autophagic machinery are an important biologic pathway in chronic inflammatory diseases of the digestive tract. One possible hypothesis is that alterations in an individual's intracellular processing of bacteria impact how the innate immune system interacts with gut microflora and may contribute to the pathogenesis of CD. Further understanding of the relevant autophagic processes, including elucidation of the molecular mechanisms by which *ATG16L1* and *IRGM* variants contribute to CD susceptibility, will have significant impact on our understanding of IBD.

Impaired Clearance of Microbes in IBD

One of the etiologic theories on the pathogenesis of IBD is impaired clearance of foreign material leading to a sustained activation of monocytes and a compensatory induction of adaptive immune response [33]. The compensatory activation of adaptive immune response is temporally different from the initial acute inflammatory response and leads to perpetuation of the chronic inflammatory response contributing to IBD. The immunodeficiency phenotype is also consistent with the finding of *CARD15* as a CD susceptibility gene [34] and further discussed below. *CARD15* encodes NOD2, which is a general sensor of bacteria that recognizes muramyl dipeptide (MDP), a component of both gram-positive and gram-negative bacteria [34]. The main outcomes of NOD2 activation is the induction of nuclear factor (NF)- κ B and the mitogen-activated protein kinase (MAPK) pathway resulting in the production of proinflammatory mediators [34]. Peripheral-blood mononuclear cells isolated from CD patients who carry the risk *CARD15* allele show a defect in IL-1 β secretion [35]. Additionally, epithelial cells transfected with 3020insC *CARD15* mutation have defective NF- κ B activation upon MDP stimulation [36,37]. The defective induction of proinflammatory cytokines may result in impaired mucosal host clearance of bacteria, as seen in NOD2-deficient mice that develop worse liver infection to *L. monocytogenes* [38]. The findings that CD variants in the autophagy genes *ATG16L1* and *IRGM* result in reduced clearance of bacteria also support the idea that impaired clearance of microbial organism from bowel wall causes IBD [30,32].

Recruitment of neutrophils to sites of trauma in the bowel is impaired in CD patients [33]. This finding led to the hypothesis that delayed neutrophil recruitment impairs the clearance of microbial organisms, which then leads to gut inflammation. This hypothesis was recently tested by injecting heat-killed *Escherichia coli* into the forearm of human CD, UC, and healthy control subjects [39•]. The investigators found accumulation of neutrophils at the site of injection as well as the clearance of injected *E. coli* was markedly impaired in CD patients [39•]. Together, these data support the notion that impaired clearance of antigenic material contributes to the pathogenesis of IBD.

Adaptive Immunity

Recognition of commensal-derived antigens by the adaptive immune system or its stimulation by the innate immune system plays a key role in the pathogenesis of IBD. Polymorphisms of genes involved in the Th1, Th2, and Th17 immune pathway have been shown to be associated

with IBD, with CD having predominately a Th1 and/or Th17 cytokine profile and UC a Th2 and, recently, a Th17 cytokine profile.

TL1A (*TNFSF15*) Bridges Innate and Adaptive Immune Response in IBD

TL1A (*TNFSF15*), an IBD-associated gene, is a member of the tumor necrosis factor (TNF) superfamily that binds to death domain receptor 3 (DR3, *TNFRSF25*), and its expression is increased in inflamed tissue of colon and small bowel of CD patients and colocalizes to macrophages and T cells [1]. In particular, lamina propria, but also peripheral CD4⁺CCR9⁺ T cells, constitutively express membrane TL1A and are especially sensitive to TL1A stimulation [1]. The role of TL1A in the pathology of mucosal inflammation was recently tested by using neutralizing antibodies to TL1A where inactivating the function of TL1A leads to attenuation of inflammation in two different murine colitis models [40••].

GWAS have revealed a significant association of genetic variants of the *TL1A* (*TNFSF15*) gene with CD in a large cohort of Japanese patients, in several European cohorts [11,41], in US Jewish patients [42], and combined data from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) IBD Genetics Consortium, Belgian-French IBD Consortium, and the Wellcome Trust Case-Control Consortium (WTCCC) [3••]. *TL1A* is the only gene that has been associated with both Asian and Caucasian IBD. Using case-control and family-based study designs, *TNFSF15* *haplotype A* has been found to confer significant CD risk whereas *haplotype B* confers protection in Asian and European populations [3••,11,43]. However, a recent report demonstrated that *TL1A* *haplotype B* is a risk *haplotype* in Jewish CD patients with antibody titers for the *E. coli* outer membrane porin C (OmpC) [44]. Moreover, CD14⁺ monocytes isolated from Jewish OmpC⁺ patients homozygous for *TL1A* gene *haplotype B* express higher levels of TL1A in response to FcγR stimulation [44]. These results show that TL1A genetic variations contribute to exacerbated induction of TL1A, which may lead to dysregulated immune response and chronic inflammation. Further work is needed to determine the exact TL1A disease-associated variants that will allow us to further explain the complicated genotype/functional correlations in these different ethnic groups.

We previously showed that TL1A is induced by the FcγR signaling pathway [45]. Recently, the potential interaction of TL1A in microbial-host interaction was illustrated by a report showing microbial organisms can induce TL1A in APCs [46]. Microbial-activated TL1A was in part mediated by TLR 1, 2, 4, and 9 signaling pathways and dependent on downstream p38 MAP kinase and NF-κB activation (Fig. 1) [46]. Negative regulators of the TL1A signaling pathway also exist and may serve to maintain gut immune homeostasis. Our group showed that TLR8 or TLR7/8 ligand (R848) can inhibit TL1A production in a dose dependent manner [47•]. Therefore, TLR8 signaling pathway may represent a novel therapeutic target in IBD.

TL1A plays an important role in modulating adaptive immune response (Fig. 1). In the Th1 effector arm, TL1A was found to augment IFN-γ production by IL-12/IL-18-stimulated CD4⁺/CCR9⁺ T cells that are specifically enriched in the intestinal immune compartment [1]. Furthermore, in autologous monocyte-T-cell co-cultures, TL1A production by monocytes potentiated IFN-γ production by CD4⁺ T cells [45,46]. In addition to mediating Th1 response, the role of TL1A in Th2-mediated functions and disease pathology was demonstrated in a mouse model of allergic lung inflammation where TL1A signaling is required to exert Th2 effector function in Th2-polarized CD4 cells and co-stimulate IL-13 production by activated NK T cells [48]. Additionally, antibody blockade of TL1A inhibits lung inflammation and production of Th2 cytokines such as IL-13. Together, this study implicates TL1A in augmenting Th2 effector function [48]. Using TL1A-deficient mice, another report showed that TL1A^{-/-} dendritic cells exhibited a reduced capacity in supporting Th17 differentiation and proliferation [49]. Consistent with these data, TL1A^{-/-} mice display decreased clinical

severity in experimental autoimmune encephalomyelitis, a Th17-mediated autoimmune disease model [49]. Furthermore, IFN- γ and IL-17 production induced by IL-12 and -23 respectively, can be synergistically enhanced by TL1A [40•,49]. Interestingly, a report using DR3-deficient mice showed that TL1A/DR3 signaling is dispensable for polarization of naïve CD4⁺ T cells into Th1, Th2, or Th17 effector cell subtypes [50]. Instead, DR3 expression is required on T cells for immunopathology, local T-cell accumulation, and cytokine production, suggesting that TL1A/DR3 signaling is important to co-stimulate antigen-induced expansion of primed T cells in the target organ of T cell-mediated autoimmune and inflammatory diseases.

Together, TL1A/DR3 signaling appears to have pleiotropic effects that include amplifying the innate immune response, modulating adaptive immunity by augmenting Th1, Th2, and Th17 effector cell function, and T-cell accumulation and immunopathology of inflamed tissue (Fig. 1). Given its immune modulatory effects, blocking TL1A-DR3 signaling is a promising therapeutic strategy in a variety of T cell-dependent autoimmune diseases, including IBD.

Conclusions

The recent GWAS and the identification of additional susceptibility UC loci demonstrate that the pathophysiology of CD and UC involves overlapping and divergent pathways. One of the new findings is the shared immunopathophysiologic pathway between IBD subtypes, particularly the IL23/Th17 inflammatory axis. In addition, these genetic findings have further implicated both innate and adaptive immune dysregulation as risk factors for developing IBD. These immunogenic parameters are biologically divergent, which explains the heterogeneous clinical manifestations and lack of a universal therapeutic response to any single agent. The challenge for the future is to correlate IBD susceptibility loci to molecular pathways and clinical phenotypes. These multidisciplinary approaches are likely to stratify this complex disease into distinct subgroups that are more biologically homogenous with more defined pathogenic parameters and aid in the evaluation of IBD susceptibility genes.

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Figure 1.

Working hypothesis of inflammatory bowel disease. Intestinal immune system is in close apposition to luminal antigen/bacteria, which are separated by a single layer of epithelial cells. Goblet cells contribute to the formation of the protective mucus layer, and M cells and dendritic cells (DC) sample intestinal luminal contents. Overresponse to antigens, either through Toll-like receptors (TLR), intracellular sensor NOD2, or antigen processing via autophagy, results in stimulated DC that recruit and generate T-cell (Th1, Th2, and Th17) and NK-cell subtypes. One subpopulation of RORγt⁺NKp46⁺ NK cells that resides in gut cryptopatches is found to readily produce IL-22 in response to IL-23, likely released by DC. IL-22 may play an important role in epithelial homeostasis, bacteria clearance, and tissue repair. TL1A appears to be a critical factor important in generating Th1, Th2, and Th17 cells. For each T-helper cell differentiation program, specific transcription factors and cytokine milieu are required (indicated in the figure). Terminally differentiated T-helper cells are characterized by a specific combination of effector cytokines (indicated in the figure) that orchestrate effector function of the adaptive immune system. Crohn's disease is a predominantly Th1- and Th17-mediated process, whereas ulcerative colitis appears to be mediated by Th2 and possibly Th17.

Table 1

Summary of design, population, and findings in eight inflammatory bowel disease genome-wide association scans and meta-analysis

Study and population	SNPs analyzed	Sample size: cases-controls	Phenotype associated	Genes identified	Loci identified
Yamazaki [11]; Japanese and British Caucasian	72,000	94-752*	CD and UC	<i>TLIA(TNFSF15)</i>	
Duerr et al. [4]; North American Caucasian	304,000	946-977	Ileal CD	<i>IL23R</i> <i>ATG16L1</i> <i>PHOX2B</i> <i>FAM92B</i> <i>NCF4</i>	Chromosome 10 [§]
Hampe et al. [7]; German Caucasian	7000 [†]	735-368	CD	<i>ATG16L1</i>	
Libioulle et al. [8]; Belgian/French Caucasian	302,000	547-928	CD	<i>IL23R</i>	5p13 [‡]
Wellcome Trust Case-Control Consortium [2]; British Caucasian	469,000	1748-2938	CD	<i>IL23R</i> <i>ATG16L1, IRGM</i> <i>NKX2-3</i> <i>PTPN2</i>	Chromosome 3 [§] 5p13 [‡]
Franke et al. [6]; German Caucasian	92,000	393-399	CD	<i>NELL1</i>	5p13 [‡]
Raelson et al. [9]; Quebec Caucasian	164,000	382 trios	CD	<i>IL23R</i>	Chromosome 17 [§]
Fisher et al. [5]; British Caucasian	10,886 [¶]	905-1465**	UC	<i>ECM1</i> <i>MST1</i> <i>BTNL2</i> <i>HLA-DRB1</i>	
Barrett et al. [3••]; North-American, British, and Belgian/French populations	NA	3230-4829		<i>IL23R</i> <i>ATG16L1, IRGM</i> <i>MST1</i> <i>PTGER4</i> <i>TLIA(TNFSF15)</i> <i>ZNF365</i> <i>NKX2-3</i> <i>NOD2</i> <i>PTPN2, PTPN22</i> <i>ITLN1</i> <i>IL12B</i> <i>CDKALI</i> <i>CCR6</i> <i>C11orf30</i> <i>LRRK2</i> <i>MUC19</i> <i>STAT3, JAK2</i> <i>ICOSLG</i> <i>ORMDL3</i>	5q31 [§] 1q24 [§] 1q32 [§] 6q21 [§] 7p12 [§] 8q24 [§] 10p11 [§] 13q14 [§] 21q21

Study and population	SNPs analyzed	Sample size: cases–controls	Phenotype associated	Genes identified	Loci identified
Frankel et al. [12]; European population	440,794	1167–777	UC	<i>HLA-DRA</i> <i>BTNL2</i> <i>IL23R</i> <i>ARPC2</i> <i>IL10</i> <i>JAK2</i> <i>SI00Z</i>	
Silverberg et al. [10]; European Caucasian	280,748	1052–2571	UC	<i>IL22</i> <i>IL23R</i> <i>IL26</i> <i>MHC</i> <i>PLA2G2E</i> <i>IFN</i>	1p36 1p31 6p21 12q15

* Study performed in 94 cases and 752 controls as stage 1. Findings reproduced in 490 Japanese cases and 345 controls, 347 British inflammatory bowel disease trios, and 363 cases and 372 controls.

† These were all nonsynonymous (amino acid changing) polymorphisms.

‡ Several groups identified association with a variant in a gene desert on chromosome 5p13. By using an expression database, this region may be involved in regulation of the prostaglandin E receptor 4 gene (*PTGER4*).

§ Several studies identified association with genetic regions in which it was unclear which was the specific causative gene.

¶ Nonsynonymous and MHC tagging SNPs.

** Study performed in 905 UC cases and 1465 controls. Positive findings tested in 936 UC cases and 1470 controls.

CD—Crohn’s disease; MHC—major histocompatibility complex; NA—not applicable; SNP—single nucleotide polymorphism; UC—ulcerative colitis.