HHS Public Access

Author manuscript

Inflamm Bowel Dis. Author manuscript; available in PMC 2018 September 30.

Published in final edited form as:

Inflamm Bowel Dis. 2015 May ; 21(5): 1166–1175. doi:10.1097/MIB.0000000000000329.

Very Early-onset Inflammatory Bowel Disease: Gaining Insight Through Focused Discovery

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Abstract

The pathogenesis of pediatric inflammatory bowel disease (IBD) is only partially understood. Strong evidence implicates a strong genetic component including high monozygotic twin concordance and familial disease phenotype concordance rates. Genome-wide association studies have identified associations between >160 genetic loci and the risk for developing IBD. The roles of implicated genes are largely immune-mediated, although other functions include cellular migration, oxidative stress, and carbohydrate metabolism. Additionally, growing literature describes monogenic causes of IBD that frequently present as infantile or very early-onset IBD. The interplay between IBD risk single nucleotide polymorphisms and rare genetic variants has yet to be determined. Studying patients with very early-onset IBD may elicit genetic factors that could be applied to broader populations of IBD. This review describes what is known about the genetic causes of very early-onset IBD and genetic strategies that may unravel more of the genetic causes of IBD.

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Author contributions: C. J. Moran, C. Klein, A. M. Muise, S. B. Snapper participated in the writing of the manuscript.

The authors have no conflicts of interest to disclose.

Keywords

Crohn's disease; ulcerative colitis; inflammatory bowel disease; genetics; very early onset IBD; immunodeficiency; pediatric; genome-wide association studies; whole exome sequencing

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract that can present at any age.[1] IBD encompasses 2 disease entities, ulcerative colitis (UC) in which the inflammation is limited to the large intestine and Crohn's disease (CD) where the inflammatory pattern typically involves the small and/or large intestine but can affect any segment of the gastrointestinal tract. The initial evidence supporting a genetic cause for IBD is provided by the high concordance rates for CD in monozygotic twins and the increased risk of children with parents who suffer from IBD.[2–7] However, despite decades of inquiry with advancing technology, the currently known genetic factors contribute only 20% to 25% of disease heritability; an understanding of the underlying genetic trigger(s) remains elusive.[8,9]

Children constitute a significant proportion of patients with IBD, with pediatric-onset IBD comprising 25% of IBD population in the United States.[10,11] In contrast to adult-onset presentations, children with UC are likely to present with pancolitis, whereas children with CD more frequently present with ileocolonic disease and only rarely present as isolated ileal disease.[12–15] Those children diagnosed with CD in the first 8 years of life usually present with isolated colonic inflammation (rather than ileitis or ileocolitis).[15–18] Due to the frequent colonic presentation of very young patients with CD, a higher percentage of these patients may be inaccurately classified as UC during the initial evaluation.[19] Children with IBD are more likely to receive corticosteroids, be initiated on immunomodulators, and require surgery in the first year after diagnosis than adults with IBD.[13,20,21]

Concomitant with the growing interest in studying pediatric-onset IBD, subclassifications of pediatric populations that reflect inherent distinctions in phenotypic and genotypic patterns have been developed. The Montreal Classification for IBD categorized all pediatric patients (diagnosed at <17 yr of age) in a single group.[22] The subsequent Paris Modification divided pediatric-onset IBD into 2 groups: A1a (diagnosed <10 yr of age) and A1b (diagnosed between 10 and 17 yr of age), although these new pediatric categories may not reflect intrinsically different disease processes.[23] As mentioned above, a notable phenotypic difference in very young patients with CD is the predominantly colonic involvement, contrasting with the increasing occurrence of ileal involvement starting at 8 years of age.[16–18,24] In addition, specific serologic patterns (CBir1 positivity in patients with otherwise negative serology) are associated with children diagnosed with CD <8 years of age.[25] The fundamental differences between older A1a subjects and younger A1b may be quite small, and the disease present in many of these patients may behave similar to that of adult-onset IBD. Therefore, better classification schemes reflecting differences in pathophysiology and genetics are desirable.

Perhaps a more distinct population are patients with very early-onset IBD (VEO-IBD) who have disease onset in the first 6 years of life, which constitute 4% to 10% of pediatric IBD.[16,26] By nature of their earlier diagnosis, patients with VEO-IBD will likely have longer

exposure to immunosuppressive medications, more surgical interventions over their lifetime, and possibly an overall more complicated disease course. Limited series of infantile-onset IBD suggest that they often have severe presentations, and the courses are complicated by serious infections raising concerns of immunodeficiency.[27] However, recent evidence shows that patients with VEO-IBD do not seem to have a more aggressive phenotype compared with adolescent onset IBD.[28] Similarly, although patients with VEO-IBD are more likely to have a family history of IBD, and there exists a growing literature of individual cases with Mendelian IBD, it is unclear whether the genetic influences in this population as a whole are unique.[29,30] In this regard, further studies are needed to determine whether the natural history and genetic susceptibility in patients with VEO-IBD is distinct from the more common presentation of IBD in older children. We believe that these patients may offer the unique opportunity to determine the role of genetic susceptibility in a context where chronic environmental influences may be less important, and at the very least, develop a better framework for studying this complex disease. This review will describe the overall pattern of genetics in VEO-IBD (in the context of pediatric IBD overall) and highlight some of the rapidly expanding areas of study.

COMMON POLYMORPHISMS ASSOCIATED WITH IBD RISK

Early work to identify genetic causes of IBD largely focused on genetic linkage analyses. Initially, genetic risk was found in major histocompatibility complex Class II molecules with both UC (DR2, DR9, and DRB1*0103) and CD (DR7, DRB3*0301, and DQ4).[31–34] Linkage studies requiring large pedigrees enriched for IBD led to the discovery of the CD risk loci of IBD1 and IBD5.[35,36] *NOD2*, a key pattern recognition receptor in innate immune and epithelial cells, that recognizes microbial products was ultimately implicated at the IBD1 locus, illustrating a pivotal role of the crosstalk between intestinal microbiota and the innate immune system in IBD pathogenesis.[37–39] Three single nucleotide polymorphisms (SNPs) in *NOD2* are linked to the majority of risk seen with the IBD1 locus (with a variety of other SNPs being less commonly reported), and increased risk has been demonstrated in both heterozygotes and homozygotes for these *NOD2* SNPs.[40–42] Such linkage studies facilitated the discovery of high-impact CD variants whose significance has remained in the genome-wide association era.

Genome-wide association studies (GWAS) have rapidly expanded our knowledge of the role of common genetic variants in complex genetic disease pathogenesis. Currently, over 160 loci have been associated with IBD with most loci contributing to both CD and UC risk; however, some variants are unique to CD- or UC-specific risk (Tables 1 and 2).[43,44,52] GWAS have identified SNPs associated with IBD risk that are in loci of genes involved in many immune-related pathways such as autophagy (*ATG16L1, IRGM, LRRK2*), adaptive immunity (*IL2, IL12B, IL23R*), and immunoregulation (*STAT3, TYK2, IL10)*.[43,44,53,57–59] Additionally, these studies have highlighted risk associated with other relevant pathways including the maintenance of epithelial integrity (*MUC19, CDH1*), antigen presentation (*ERAP2, DENND1B*), and endoplasmic reticular stress (*CPEB4, ORMDL3*).[46–49,51,52,54–56,60] Furthermore, deep resequencing of risk loci (e.g., *NOD2*, *CARD9*, *IL23R*) have identified additional and perhaps more functionally significant variants independent of the original SNPs found by GWAS.[61,62]

Not only have GWAS of adult IBD reaffirmed the risk of *NOD2* with CD, but they have identified specific clinical phenotypic presentations that are associated with *NOD2* SNP carriers (ileal involvement and surgical resection).[53,63,64] GWAS have identified additional genetic factors for colonic involvement of CD (*ZPBP*) and ileocolonic involvement of CD (*JAK2, IL23R*).[65] Variation in genes involved in autophagy (*IRGM, ITLN1*, and *ATG16L1*) has been associated with ileal-predominant CD (as well as upper gastrointestinal tract involvement).[66,67] Patients with CD who have a higher genetic burden (of the 140 SNPs associated with CD) have an earlier age of onset and are more likely to have ileal involvement.[68]

A complimentary approach to classic GWAS strategies are pathway/network analyses, which have led to a better understanding of the pathogenesis of type 2 diabetes, obesity, pancreatic and bladder cancer, psoriasis, lymphoma, and Behçget's disease.[69–74] These methods often rely on assignment of SNPs to a specific gene and specific tissue expression pattern information — tasks not always possible— and can benefit from some knowledge of gene function.[75,76] Pathway and network analyses using data from adult-onset IBD GWAS have confirmed the involvement of known immune-mediated signaling pathways (IL-12, interferons) and antigen presentation but have also highlighted novel immune (activation of IL-9 and IL-2Rβ), and non-immune pathways (lipid metabolism).[43,77,78] In addition, approaches agnostic to gene function have identified broader Nod2-focused IBD causal subnetworks that involve genes enriched in anti-inflammatory macrophages.[43]

GWAS and pathway/network analyses of adult-onset IBD have further emphasized that the pathophysiology of IBD likely results from either innate and/or adaptive immune defects although most SNPs detected in IBD GWAS have relatively modest effect sizes (with the exceptions of *NOD2* and *IL23R*).[52] However, identified variants may have stronger effects on specific IBD subsets (as discussed above for particular disease locations) and for pediatric-onset IBD. Regarding pediatric IBD pathogenesis, studies of adult cohorts may have overlooked some of the key genetic factors in pediatric IBD simply because younger patients were not included in initial GWAS. Furthermore, given that it is sometimes difficult to associate an SNP with a particular gene, the mechanistic explanation of the identified disease risk can be difficult to ascertain.

PEDIATRIC PERSPECTIVE OF IBD GENETICS

The SNPs identified initially by GWAS in adult IBD were based on cohorts with patients that have been drawn from both young and older adult patients with IBD. Multiple studies have demonstrated that many adult-onset CD SNPs (including *IL23R, NOD2*, and *LRRK2*) play a role in pediatric-onset CD, although these cohorts predominantly included teenage-onset (A1b) CD.[45,79] Initial GWAS that included only pediatric-onset IBD replicated associations with 8 of 17 adult-onset UC SNPs (including IL-10).[45,50] However, the results from GWAS of adult IBD may not be easily extrapolated to represent the risk in significantly younger subjects because they were largely missing from these studies.

In addition to replicating SNPs associated with adult-onset IBD, pediatric IBD GWAS identified novel SNPs not found in initial adult-onset IBD GWAS. Imielinski et al[45] reported

associations between pediatric CD and SNPs in the *interleukin 27 (IL-27), ZMIZ1*, and *MTMR3* loci as well as an association of the *CAPN10* locus with pediatric UC. IL-27 induces type 1 regulatory T cells and suppresses T_H17 cells, and the *IL27* risk SNP results in a 7-fold reduction of IL-27 production from lymphoblastoid cells.[45,80] Kugathasan et al[50] identified *TNFRSF6B* and *PSMG1* loci as SNPs that confer risk for pediatric IBD. *TNFRSF6B* encodes DCR3, which is a soluble receptor that modulates the differentiation of dendritic cells and T cells and alters cellular sensitivity to FasL-induced death, whereas PSMG1 is critical in the formation of the 20S proteasome.[81–84] Subsequently, the *IL-27, MTMR3, TNFRSF6B, PSMG1*, and *CAPN10* associations were detected in the large-scale metaanalyses of adult CD and UC.[43,44,52] Interestingly, a *ZMIZ1* SNP (rs1250550) had a protective effect in pediatric CD, whereas this SNP and others within the region were found to confer increased risk for adult-onset CD.[45,52] These data indicate that many SNPs perceived to be pediatric-specific were ultimately found in the large-scale meta-analyses of adult IBD studies, although the effect sizes for some of these SNPs is different than that of their adult IBD counterparts.[43,44,52] Similarly, GWAS-based pathway/network analyses that have included pediatric cohorts have identified pathways (e.g., IL-12/IL-23 signaling) in pediatric IBD that have also been identified in adult.[43,85]

GWAS assume that common yet complex diseases are the result of “common” polymorphisms. It is notable that 113 of the 163 IBD SNPs are also found to enhance risk for other autoimmune diseases (including lupus, rheumatoid arthritis, and ankylosing spondylitis), suggesting a partially shared pathogenesis.[43] Early GWAS were powered to identify disease risk associated with SNPs with a minor allele frequency of >5%.[86] Recent meta-analyses of GWAS data (benefiting from larger samples sizes) have lowered the detection threshold to ~1%. Patients with disease caused by highly penetrant, loss-of-function rare variants (with minor allele frequency <1%) are likely overlooked by current GWAS strategies. SNPs found by GWAS to be associated with IBD risk alone are unlikely to be independently causative given the high prevalence of these SNPs in nondiseased individuals.[44,52] Additionally, although these SNPs are associated with risk for IBD, these specific SNPs may not be specifically responsible for the risk but rather they may be in strong linkage disequilibrium with the responsible genetic variant.

Moreover, the current collection of disease-associated SNPs can best explain 20% to 25% of the heritability of IBD.[8,9] The applicability of GWAS is also a product of the study population. GWAS that involve largely adult populations of patients with IBD may overlook key regulators of pediatric IBD pathogenesis due to the absence of pediatric patients in their study cohorts. Because GWAS of pediatric IBD include predominantly adolescent-aged subjects, the applicability to patients with VEO-IBD may be limited. The study of these unique patients would be better served by a more focused approach that would be an important complement to GWAS.

LESSONS FROM PRIMARY IMMUNODEFICIENCY

Although many patients with IBD exhibit a proinflammatory state, the genetics underlying this immune dysregulation are highly variable which creates substantial obstacles to studying the immune physiology. Patients with primary immunodeficiency syndromes offer

an alternative starting point by examining gastrointestinal pathophysiology in patients with well-defined and more uniform immune defects to observe the downstream defects. Chronic granulomatous disease (CGD), Wiskott—Aldrich syndrome (WAS), Immune dysregulation-Polyendocrinopathy-Enteropathy-X-linked syndrome, glycogen storage disease Type 1b, NEMO deficiency, and Hermansky—Pudlak syndrome all may show varying degrees of gastrointestinal symptoms.[87–91] Studying well-defined immune syndromes associated with gastrointestinal inflammation can act as an important complement for studying a complex genetically mediated disease such as IBD.[30]

WAS is an X-linked condition that is characterized by the classic triad of recurrent infections, thrombocytopenia, and eczema due to defective Wiskott-Aldrich Syndrome protein (WASp) expression.[91] Mice deficient in WASp develop severe T_H2-predominant colitis in part due to defective regulatory T-cell (Treg) function and aberrant antigen presenting cell function.[92–94] However, less than 10% of patients with WAS develop intestinal inflammation.[95] Disease severity correlates with the degree of WASp dysfunction, as patients who possess mutations that result in full-length WASp with only altered amino acid sequences can develop a thrombocytopenia syndrome (X-linked thrombocytopenia) without eczema or recurrent infections, whereas mutations leading to truncated WASp often lead to classic WAS.[96] Whether the incomplete penetrance of gastrointestinal symptoms in patients with WAS is due to the severity of the WASp defect, mutations in other immune-mediated genes in those patients, or a dysregulated intestinal microbiome has yet to be elucidated.

CGD is an immunodeficiency syndrome due to a defective oxidative burst from the NADPH oxidase complex.[97] The NADPH oxidative complex consists of 5 proteins: one gene on the X chromosome (*gp91^{phox}*) and 4 autosomal genes (*p47^{phox}*, *p67^{phox}*, *p22^{phox}*, and *p40^{phox}*).[90,98] X-linked CGD is more common than all forms of autosomal CGD combined and presents at a significantly younger age than autosomal CGD.[98–100] These patients develop recurrent infections from *Staphylococcus aureus, Serratia marcescens, Burkholderia cepacia, Nocardia*, and *Aspergillus* due to defective intracellular killing by phagocytes.[90,101] Nearly 50% of patients with CGD have gastrointestinal and hepatic complications, among these symptoms include colitis and gastrointestinal granulomas that resembles CD.[102,103] Gastrointestinal symptoms occur more commonly in X-linked CGD and in autosomal recessive forms of CGD that have concomitant variants in myeloperoxidase and FcRγIIIb genes.[100,104,105] Even in a well-defined immunodeficiency, genetic heterogeneity seems to modulate gastrointestinal presentations.

In a similar manner, genes involved in well-defined immunodeficiency syndromes may also contribute to states of immune dysregulation such as IBD. Patients with CD have long been described to display defects in oxidative burst, and GWAS have identified an SNP in the *NCF4* locus (*p40^{phox}*) that associates with the risk for ileal CD.[106–108] Taking a candidate gene approach, Muise et al[109] recently showed that mutations in *NCF2* (*p67^{phox}*) are associated with VEO-IBD. More recently, additional genetic variants in NADPH oxidase genes have been shown to associate with VEO-IBD.[110] These well-described syndromes described above are only but a few of the large list of immunodeficiency syndromes with both intestinal and extraintes-tinal manifestations.[30] An equally important pool of

knowledge can be gained from studying immunodeficiency syndromes with more gastrointestinal predominant symptoms.

IL-10 and genes downstream of IL-10 signaling have consistently been demonstrated by GWAS (e.g., *Tyk2; Stat3*) to be associated with IBD risk.[43,44,52] Much stronger evidence for the involvement of defective IL-10 signaling comes from the analysis of infantile-onset IBD patients.[111] Linkage studies (as well as targeted sequencing of IL-10R genes) in patients with VEO-IBD have identified homozygous mutations that lead to colitis and variable phenotypes of folliculitis and occasionally deepsited bacterial infections and polyarthritis.[112–117] Patients with IL-10 defects have a similar phenotype.[118] These patients uniformly have aggressive IBD with significant perianal involvement (whose luminal disease is largely limited to the colon) that is recalcitrant to immunosuppressive agents and surgery, requiring allogeneic stem cell transplantation for control of the disease.[112–115,117,118] Interestingly, patients with mutations in *IL10RA* and *IL10RB* are also prone to develop intestinal diffuse large B-cell lymphomas, potentially caused by aberrant antitumor T-cell responses.[119]

IL-10R genes may not have been identified in pediatric IBD GWAS cohorts due to the primarily adolescent nature of pediatric GWAS cohorts or due to the limited coverage of SNPs in these genes. Extending from these initial studies in infantile-onset IBD, we recently completed a candidate gene analysis in children with VEO-IBD, identifying an association between *IL-10RA* SNPs and very early-onset UC.[113] The expanding role of defective IL-10 signaling and the oxidative burst pathway in IBD pathogenesis illustrate the ongoing value that candidate gene analysis and linkage studies will have in studying IBD pathophysiology to pursue rare variants that elude GWAS detection.

NEW DIRECTIONS IN STUDYING EARLY-ONSET IBD

Significant progress in understanding the genetics of VEO-IBD has been made by studying families with high degrees of consanguinity. The evolving description of infantile-onset IBD patients with defective IL-10 signaling and the discovery of an IBD-like condition in patients with mutations in *ADAM17* demonstrate the benefits of SNP-homozygosity mapping and linkage analysis.[112,116,118,120] This allows for mutation discovery in consanguineous families given the high likelihood that a rare homozygous variant is present in these patients. However, these strategies may also overlook subjects who possess compound heterozygous mutations and dominant mutations with variable penetrance.

Whole exome sequencing (WES) has a developing role in the study of VEO-IBD and other rare disease processes.[121] The exome includes all coding regions that comprise approximately 1% to 2% of the human genome.[122] By using next generation sequencing, WES allows for the identification of coding variants across the exome and can play a vital role in gene discovery as most Mendelian disorders appear to result from genetic variation in the exome.[123] This technology is becoming rapidly more accessible as the cost for sequencing an individual’s entire exome decreases. Limiting factors for WES are the ability to analyze (and store) the large amount of data generated by these sequencing efforts.[124] Typically, individuals of European ancestry possess roughly 20,000 exomic variants (whereas the total in those of African ancestry averages closer to 24,000 variants), but only

~2% result in altered protein structure.[125] Utilization of the enormity of these data is facilitated by limiting the analysis to those variants that are novel and alter amino acid sequence.[124]

Initially, WES was shown as an effective tool in the study of rare, Mendelian diseases.[124] One only needs DNA samples from the probands and multiple first degree relatives to search for the causative genetic mutation. Studies using WES in Coffin–Siris syndrome, Miller syndrome, and mandibulofacial dysostosis with microcephaly have identified a number of genes not previously thought to be relevant to the underlying disease.[126–128] More recently, applications of WES in common diseases (such as Alzheimer's disease, multiple sclerosis, and familial dyslipidemia) have identified genetic variants not found by GWAS.[129–132]

The introduction of WES to the arena of IBD research led to the description of a hemizygous mutation in *X-linked inhibitor of apoptosis protein* gene (*XIAP*) in an infant with aggressive colitis.[133] XIAP is an intracellular protein that interacts with caspases, NOD2, and NFκB and is expressed in all hematopoietic cells.[134] Patients with XIAP deficiency classically develop an x-linked lymphoproli-ferative syndrome and hemophagocytic lymphohistiocytosis.[135,136] Although *XIAP*-deficient patients had previously been described to have gastrointestinal manifestations, this report was the first of several to report a primary Crohn's-like phenotype.[133,137] Further analysis of the role that *XIAP* plays in pediatric IBD have shown that patients with *XIAP* deficiency can present with pediatric CD.[138,139]

The promise of WES includes the ability to identify causative mutations in genes that are not a priori judged to be involved in disease pathogenesis. WES has identified a homozygous *PIK3R1* mutation in a patient with defective B-cell development and a homozygous mutation in *LRBA* in a patient with a common variable immunodeficiency, both of whom had colitis.140–142 Most recently, patients presenting with recurrent intestinal atresia, combined immunodeficiency, and enterocolitis have been described due to mutations in *TTC7A*.143–146 Much of the interest in WES has focused on its use in consanguineous families with extremely rare diseases, but WES has also been useful in identifying compound heterozygous mutations (e.g., *IL10RA*) in nonconsanguineous families.147

Although one obvious use of WES can be to ascertain the causative gene in extreme cases, it can also be applied to understand more common disease risk. More recently, WES was used in a cohort of patients with adult-onset CD to identify a novel risk SNP in *NDP52* and to clarify that the risk for UC and CD seen at the 6q21 locus is due to genetic variation in *PRDM1*.[148] Additionally, WES in 8 patients with pediatric-onset CD identified nonsynony-mous variants (mostly in the heterozygote state) in genes involved in IBD pathogenesis but did not identify a clear causative mutation in any of the patients.[149] Although the potential yield of studying patients with VEO-IBD from consanguineous families may be the highest for gene discovery, there remains utility in studying patients apart from this very select population. The ability to study a multitude of genes simultaneously and the ability to identify compound heterozygotes in a particular disease make WES a rapidly developing field. However, WES does not address the ability of regulatory regions in the human genome to modulate disease.

An extension to WES, whole genome sequencing (WGS), offers the ability to obtain genetic information at every locus throughout the human genome. WGS has already displayed great benefit in identifying causative mutations in various cancers (multiple myeloma, melanoma, and hepatocellular carcinoma) and rare Mendelian disorders such as metachondromatosis, as well as a recent prenatal diagnosis of CHARGE syndrome.[150–154] The application of WGS technology to more complex genetic diseases has been largely limited to individual subject analysis due to a multitude of reasons.[155] As WGS captures more data, it also carries a higher cost of sequencing and a greater burden of data analysis and storage. The ability of both WES and WGS to detect variants is dependent on the coverage of the sequence amplifiers used by each platform which also continues to improve. The current cost differential of WES and WGS may limit large-scale studies from using WGS, but accessibility to each of these technologies will increase as the cost of both continues to fall.

CONCLUSIONS

A better understanding of the genetics of IBD will undoubtedly occur from a combination of genetic strategies. Despite the significant progress made during the GWAS era, it is estimated that the currently known risk alleles only explain ~20% to 25% of the heritability of CD.[52] A substantial proportion of the remaining risk may be due to rare variants. The advent of WES (and WGS) allows for the identification of genes not previously known to be involved in IBD and subsequently allows further analysis in larger cohorts by traditional genotyping and sequencing strategies. Although some genetic variants may contribute only modestly to overall pediatric IBD, they may have stronger effects on distinct subtypes of IBD (such as VEO-IBD) and allow stratification of patients into subsets to allow for better tailoring of therapy. Furthermore, the pathways identified by GWAS can allow for a direct pathway analysis in a broader IBD cohort. This will ultimately result in identification of patients with fundamental defects of their immune system that may benefit from more aggressive alternative therapy (such as allogeneic hematopoietic stem cell therapy) rather than profound immunosuppression and repeated surgical intervention. Although the underlying genetics of IBD remain elusive, recent advances in this field prepare us for the beginning of a quickly expanding research landscape.

ACKNOWLEDGMENTS

S. B. Snapper is supported by NIH Grants HL59561, DK034854, and AI50950, the Helmsley Charitable Trust, and the Wolpow Family Chair in IBD Treatment and Research.

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TABLE 1.

Single Nucleotide Polymorphisms Associated with Ulcerative Colitis in GWAS

	Adult-Onset UC SNPs		**Pediatric-Onset and Adult-Onset UC SNPs**
ADCY3[43]	GPR65/GALC[43]	PTGER4[43,44]	CAPN10/GPR35[43–45]
ARPC2/IL-8RA/IL-8RB[44,46]	GPR183/GPR18[43]	REL/PUS10[43,44]	HLA-DQ/HLA-DR[44–47]
C1orf53[43]	HCK[43]	RELA[43]	ICOSLG[43–45]
C1orf106[44]	HNF4A/ADA[43,48]	RFTN2/PLCL1[43]	IFNG/IL-26[43–45,49]
CALM3[43]	IFIH1[43]	RORC[43]	IL-10[43–45]
CARD9[44,46]	IBD5 locus[43]	SERINC3[44]	IL-12B[43–45]
CARD11/GNA12[43]	IL-2/IL-21/EXOC3[43,44]	SLC9A3[43]	MST1[43–45]
CCDC88B[43]	IL-2RA/IL-15RA[43]	SMAD3[43]	NKX2-3[43,45]
CCL13/CCL2[43]	IL-7R[44]	SMAD7[43]	ORMDL3[43–45]
CCR6/RPS6KA2[43]	IL-18RAP/IL-1R1/IL-1R2[43,44]	SMURF1[43]	OTUD3/PLA2G2E[43,45,49]
CD6[43]	IL-23R[43,44,47]	SOCS1/LITAF[43]	PSMG1[43,50]
CD40[43]	IL-27[43]	SPRED2[43]	TNFRSF6B[43,44,50]
CD48[43]	IRF5[43,44]	SPRY4/NDFIP1[43]	ZMIZ1/ZPBP/IKZF1[43,45]
CD226[43]	IRF8[43]	STAT1/STAT4[43]	
CDH1/CDH3[48]	IRGM[43]	STAT3[43]	
CEBPB[43]	ITGAL[43]	TAB1[43]	
CEBPG[43]	JAK2[43,44]	TBD1/RPS6KB1[43]	
CISD1/IPMK[43]	JRKL/MAML2[43]	TNFAIP3[43]	
CNTF/LPXN[43]	KIF21B[43]	TNFSF15/TNFSF8[43,44]	
CREM/CCNY[43,44]	LIF/OSM[43]	TNFRSF9[43]	
CRTC3[43]	LOH12CR1[43,44]	TNFRSF14[43,44]	
CXCR5[43]	MAP3K8[43]	TNFRSF18/TNFRSF4[43]	
DAP[43,44]	MUC19[43]	TNNI2/LSP1[43,44]	
DLD/LAMB1[43,48,49,51]	NFIL3[43]	TRAF3IP2[43]	
DNMT3B[43]	NFKB1/MANBA[43]	TRIB1[43]	
DOK3[43]	NXPE1/NXPE4[43]	TSPAN14/C10orf58[43]	
EPO[43]	PHACTR2[43]	TUBD1/RPS6KB1[43]	
ERAP2/ERAP1[43,44]	PRDM1[44]	TYK2[43]	
FCGR2A/FCGR2B/FCGR3A[43,51]	PRKCB[43]	VDR[43]	
FOSL2/BRE[43]	PRKCD/ITIH4[43]	ZFP36L1[43]	
FOS/MLH3[43,44]		ZFP90[43,44]	
		ZNF831/CTSZ[43]	

TABLE 2.

Single Nucleotide Polymorphisms Associated with Crohn's Disease in GWAS

	Adult-Onset CD SNPs		Adult-Onset and Pediatric-Onset CD SNPs
ADAM30[43]	GPR35[43]	RIPK2[43]	ATG16L1[43,45,50,52–54]
ADCY3[43]	GPR65/GALC[43,52]	RORC[43]	C11orf30[45,52,54]
BACH2[52]	GPR183/GPR18[43]	SLC22A4/IRF1/IL-3[52]	C13orf31[45,52,54]
C1orf53[43]	GPX4/SBNO2[52]	SMAD3[43,52]	CCL2/CCL7/CCL13[43,45,52]
CARD9[43,52]	HCK[43]	SMAD7[43]	CCNY/CREM[43,45,52,55]
CCDC88B[43]	HMHA1/GPX4[43,52]	SMURF1[43]	FMO4[43,45]
CCR6[43,52,54,55]	IFIH1[43]	SOCS1/LITAF[43]	HLA-DQA[50,52]
CD6[43]	IBD5 locus[43]	SP140[43,52]	ICOSLG[43,45,52,54,55]
CD40[43]	IFNG[43]	SPRED2[43]	IL-10[43,45,52]
CD48[43]	IFNGR2/IFNAR1[43]	SPRY4/NDFIP1[43]	IL-12B[43,45,52,54,55]
CD226[43]	IL-2/IL-21[43]	STAT1/STAT4[43]	IL-18RAP/IL-1R1/IL-18R1/IL12RL2[43,45,52]
CD244/ITNL1[52,54]	IL-2RA/IL-15RA[43,52]	STAT3[43,52,54,55]	IL-23R[45,50,52,55]
CDKAL1[52,54]	IL-6ST/IL-31RA[43]	TAB1[43]	IL-27/CD19[43,45,52]
CEBPB[43]	IRF8[43]	TAGAP[43,52]	JAK2[43,45,52,54,55]
CEBPG[43]	IRGM[43,52,54,55]	TBD1/RPS6KB1[43]	LRRK2/MUC19[43,45,52,54]
CISD1/IPMK[43]	KIF21B/C1orf106[43,55]	THADA[52]	MST1/PFKB4[43,45,52–55]
CNTF/LPXN[43]	LACC1[43]	TNFAIP3[43]	MTMR3[45,52]
CPEB4[43,52]	LGALS9/NOS2[43]	TNFRSF18/TNFRSF4/FASLG[43]	NKX2-3[43,45,52–55]
CREB5/JAZF1[43]	LIF/OSM[43]	TNFRSF6B[43]	NOD2[43,45,52–54,56]
CRTC3[43]	LOH12CR1[43]	TNFSF11[52]	ORMDL3/ZPBP/IKZF1/IKZF3[43,45,52,54,55]
CXCR5[43]	MAP3K7IP1[52]	TNNI2/LSP1[43]	PTGER4[43,45,52–54]
DAP[43]	MAP3K8[43]	TRAF3IP2[43]	PTPN22[43,45,52,54]
DENND1B[52]	MUC1/SCAMP3[43,52]	TRIB1[43]	TNFSF15/TNFSF8/SLC46A2[43,45,52,55]
DNMT3A/DNMT3B[43,52]	NDFIP1[52]	TSPAN14/C10orf58[43]	ZMIZ1[45,52]
DOK3[43]	NFIL3[43]	TUBD1/RPS6KB1[43]	ZNF300[45,52]
EPO[43]	PHACTR2[43]	TYK2/ICAM1/ICAM3[43,52]	ZNF365[43,45,52–54]
ERAP2/ERAP1[43,52]	PLCL1[52]	UBE2D1[52]	ZPBP[45,52]
FADS1[52]	PRKCB[43]	UCN[43]	
FCGR2A/FCGR2B/FCGR3A[43]	PRDM1[52]	VAMP3[52]	
FOS/MLH3[43]	PRDX5/ESRRA[52]	VDR[43]	
FOSL2/BRE[43]	PTPN2[52–54]	YDJC[52]	
FUT2/RASIP1[43,52]	RASGRP1/SPRED1[43]	ZFP36L1[43,52]	
GCKR[52]	RBX1-EP300[56]	ZNF831/CTSZ[43]	
	REL[43]		
	RELA[43]		