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REVIEW ARTICLE

Updates in the genetics of inflammatory bowel disease

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ABSTRACT

Ulcerative colitis and Crohn's disease are chronic, relapsing, inflammatory conditions defined by their phenotypic features. Family studies have identified a clear genetic influence on disease susceptibility and expression. Molecular studies have identified specific genes that predispose individuals to the development of inflammatory bowel disease. Some of these genes are specific to either ulcerative colitis or Crohn's disease, others are common to both conditions. The identification of susceptibility genes provides insight into the mechanisms that underlie these conditions. Furthermore it raises the possibility of anticipating disease outcome and tailoring treatment for an individual.

Keywords: Inflammatory bowel disease, Ulcerative colitis, Crohn's disease, Genetics.
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INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic, relapsing, immunologically mediated disorders.

They are collectively referred to as inflammatory bowel disease (IBD). Although both diseases can usually be differentiated by clinical, endoscopic, and pathologic findings, up to 5–10% of IBD patients have mixed CD and UC characteristics and are classified as having indeterminate or non- classifiable colitis (1). IBD is frequently a disabling disease. Established therapies and newer biological treatments frequently achieve remissions, but patients remain at risk of 'flare-ups' and complications from the disease, such as an increased risk of colorectal cancer, and side effects of treatment such as steroid induced osteoporosis. IBD also decreases fecundity and successful pregnancy outcomes. Furthermore, offspring of IBD patients are at greater risk of suffering from IBD than the general population. The prevalence of IBD rapidly increased in Europe and North America in the second half of the twentieth century and is becoming more common in the rest of the world. It is very important to determine why IBD develops and to explain the distinct, but related, nature of the two major IBD phenotypes, CD and UC.

Epidemiologic observations indicate that there are strong environmental factor influences on IBD, with an increasing incidence of IBD as countries adopt a Western lifestyle (2).

Clinical trials, human genetics studies, basic science experiments and animal models, have provided new insights into the pathogenesis of IBD. The most widely held hypothesis regarding the pathogenesis of IBD is that overly aggressive

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acquired (T cell) immune response to a subset of commensal enteric bacteria develops in genetically susceptible hosts, and that concurrent environmental factors precipitate the onset or reactivation of disease. Within this hypothesis, the etiologies of these diseases remain unknown. A convenient approach to understanding the pathogenesis of IBD considers the possible mechanisms by which UC and CD might occur. This approach addresses the possibility of a specific infectious etiology and the various ways that commensal bacteria can induce chronic, immune-mediated inflammation. The complex and delicate balance required to maintain intestinal homeostasis can be disrupted by increased or decreased expression of an ever-increasing catalog of genes.

Current knowledge of inflammatory bowel disease is based on a combination of gene association studies, clinical investigations, and laboratory experiments in animals, suggesting that IBD results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host. Therefore, genetic studies highlight the importance of host–microbe interactions in the pathogenesis of these diseases (3-14).

In fact, several authors support the notion that instead of the two single entities, CD and UC, IBD harbors many different subtypes of chronic inflammatory conditions involving the small and the large bowel. Each of these subtypes would be partially determined by a cluster of susceptibility genetic factors, and the final clinical phenotype in each individual patient, including disease location and behavior, would also be influenced by a number of disease-modifying genes and environmental factors. In this review, we discuss the evidence of a genetic role in IBD patients and genetic studies in Iran.

THE ROLE OF IBD GENETICS

Despite the obvious clinical, endoscopic, and pathologic differences existing between CD and UC, both entities have more in common than we usually suspect, and the overlap between them starts indeed at the most basic level, the genetic predisposition to suffer these conditions. An alternative perspective enables us to consider genetic factors that are specific for each condition and may unveil distinct molecular mechanisms responsible for unique features of CD and UC.

Advances have occurred in understanding the genetics of human IBD, from studies based on single nucleotide polymorphisms (SNPs) and candidate gene approaches to studies in experimental mice that used transgenic and deletion (knockout) techniques (15, 16). Work in these two independent systems has provided independent results, implicating several ‘candidate’ genes in both IBD and experimental colitis. The primary goal of genetic studies in IBD is the identification and prioritization of genetic mechanisms that contribute to the expression of IBD. The identification of key genes associated with pathogenic mechanisms may serve to prioritize development of new therapeutic approaches in IBD.

A second, related goal of IBD genetics studies is the development of predictive risk models, both in unaffected relatives of IBD probands and in predicting disease outcomes in patients with diagnosed IBD. While numerous IBD genetic studies have tested for statistical interactions between established susceptibility genes, at present, definitive interactive effects have not been established.

The common threads are that the implicated genes regulate several important biologic functions, including immunoregulation, mucosal barrier integrity, and microbial clearance and/or homeostasis. Defining the genetic basis of IBD will be a major step in determining those at greatest risk for developing IBD and will uncover pathophysiological mechanisms to apply focused interventions for prevention and treatment. It may help predict disease course and complications, and

clarify the underlying nature of IBD type unclassified or "indeterminate colitis." Additionally, far too many patients with seemingly classic UC undergo "curative" proctocolectomy only to later develop CD. Increased familial clustering and especially increased monzyogotic vs. dizygotic twin concordance predicted the existence of IBD susceptibility genes. Orholm and colleagues observed that the increased risk of developing CD in relatives of CD patients is 10-fold greater than that of controls, and for UC 8-fold greater, whereas the cross disease risk (CD in a relative of a UC patient and vice-versa) is 2-fold and 4-fold, respectively (17). This suggested that some genes would be specific to CD, others to UC, but some would be common to both types of IBD. Indeed, "mixed" IBD relative pairs (e.g., CD-UC relative pairs) are relatively common, comprising one-fifth to one-third of all IBD pairs collected for genetic linkage studies. In any case, there is little doubt that genes play a common key role in UC and CD pathophysiology (18). The observation of an increased risk to develop IBD in first-degree relatives as well as in monozygotic twins, as compared with dizygotic twins, along with the increased prevalence of IBD in subjects of certain ethnic origin, such as the Ashkenazi Jews, led to the notion that genetic predisposition is required to develop IBD (19). This concept was further supported by the identification of a number of IBD risk loci and the description of the *NOD2/CARD15* variants as the first genetic determinant of CD susceptibility.

GENETIC EPIDEMIOLOGY OF IBD

IBDs are genetically complex disorders, although there are some conservative familial patterns. For CD, approximately 15 % of patients have a family member with the disease. If one member of a pair of monozygotic twins has CD, then the other twin has a 44 % risk of developing the disease. Furthermore the disease is likely to be phenotypically similar to the 'index' affected twin. For UC the genetic influence appears to be less strong, 15 % of patients diagnosed have a family member with UC.

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Epidemiologic data from genome-wide association studies have identified loci associated solely with CD or UC and other loci associated with both disorders. Detailed sequencing studies and functional analyses are needed to identify the specific SNPs causing disease. It is estimated that known genetic associations account for only about 20% of the genetic variance underlying susceptibility to inflammatory bowel disease; the remaining genetic factors have not been identified (20- 22).

THE *NOD2* OR *CARD15* GENE

The *NOD2* gene (also known as *CARD15* (caspase recruitment domain family) is located on chromosome 16q12, and the encoded intracellular protein activates nuclear factor kB and mitogen-activated protein kinase pathways. These pathways are responsible for a bacterial recognition and an inflammatory response.

Stimulation by components of peptidoglycan (minimal bioactive component, muramyl dipeptide), present on the cell wall of gram-positive and gram-negative bacteria activates the pathway (23-25). Three variants, Arg702Trp, Gly908Arg, and Leu1007fsinsC, are each independently associated with an increased risk for ileal-only and ileocolonic CD, but not colonic-only CD or UC (26-28).

A recent meta-analysis of all previously reported association studies in European ancestry cohorts estimated that homozygous or compound heterozygous carriage of *NOD2* risk alleles confers a 17.1-fold (95% confidence interval: 10.7–27.2) increased risk of CD, whereas heterozygous carriage of *NOD2* increases the risk of CD 2.4-fold (95% confidence interval: 2.0 –2.9) (29). These odds ratios represent some of the most significant risk alleles among complex, multi-genic disorders. However, it is estimated that among *NOD2* homozygote/compound heterozygote carriers, less than 10% of individuals will manifest CD, an estimate of the genetic penetrance of the *NOD2* mutations (30, 31). In addition to the three major CD-associated mutations, a number of very rare amino acid polymorphisms either within or near the C-terminus leucine rich repeat peptidoglycan-sensing domain have been reported that likely contribute to CD susceptibility (32). Mutations in *CARD15* are associated with distal ileal CD in particular and have been found in some patients with stricturing disease.

The three major CD mutations are not observed in Asian patients with CD and uncommonly in black patients with CD (33).

There are three mutations causing amino-acid substitutions Arg702Trp and Gly908Arg and the frameshift 1007fs. These are found within the region of *CARD15* that encode a leucine-rich repeat. At least one of these mutations is present in 25–35% of CD patients of European ancestry, but not in Asian or African American CD patients (34). In a study in Iran the R702W mutation of *CARD15* gene was associated with CD (35).

Both transfection studies of mutant and wild-type *NOD2* as well as studies in primary human cells stratified by the *NOD2* genotype demonstrated decreased inflammatory responses with acute, short-term muramyl dipeptide stimulation (27, 36). By contrast, the murine knock-in of the frameshift mutation demonstrated increased nuclear factor _B activation (37); additional studies will be required to reconcile differences between the human and murine findings. If the CD-associated *NOD2* mutations represent loss-of-function mutations, the *NOD2* knockout represents an important model for assessing the functional consequences of *NOD2* polymorphisms. The *NOD2* knockout does not spontaneously develop ileitis (38, 39), demonstrating the requirement for other genetic variants and environmental and developmental factors for disease expression. Potential mechanisms of disease pathogenesis include altered cytokine regulation (40, 41), dysregulated killing of intracellular bacteria (42), and decreased cryptdin expression observed in *NOD2*-deficient mice (39). The latter observation corresponds with decreased-defensin expression observed in human CD (43) especially pronounced in individuals with *NOD2* mutations. The *NOD2* discovery provides specific support for the long-held hypothesis that CD results from a genetically dysregulated host immune response to luminal bacteria (44).

The binding of MDP by dimerized *NOD2* activates nuclear factor (NF)κB, which forms part of a central signaling pathway that stimulates the transcription of multiple genes that encode both pro-inflammatory and protective molecules. The mutations causing Arg702Trp, Gly908Arg, and 1007fs cause defective MDP binding, but studies report conflicting consequences of having such mutations. Mutant *NOD2* fails to clear Salmonella from epithelial cells (8), and clearance of invasive

bacteria is dependent on NFκB activation via the cell-death regulatory protein GRIM-19 (28, 45, 46). It is also possible that defective *NOD2* results in increased luminal bacterial populations, particularly within the crypts (47).

NOD2 is constitutively expressed in Paneth cells, (45) the source of secreted antimicrobial peptides such as the α-defensins. Targeted deletion of NOD2 in mice decreases α-defensin production and enhances susceptibility to experimental Listeria monocytogenes infection after oral, but not systemic (intraperitoneal), challenge (38). These results are consistent with the decrease in α-defensin production seen in CD patients, particularly those with *NOD2* mutations (46). In addition, Paneth cells are selectively expressed in the ileum, perhaps accounting for the distal ileal involvement of CD in patients with NOD2 mutations. Finally, Strober and colleagues have attempted to reconcile the observed activation of NFκB in patients with active CD, rather than decreased activity predicted by a loss-of-function mutation. Their investigations demonstrated that, in NOD2-defective cells, Toll-like receptor 2 (TLR2) was unable to down-regulate NFκB activation (40).

These abnormalities could result in defective down regulation of the innate immune response to bacterial adjuvant stimulation, ineffective clearance of intracellular bacterial infection, and proliferation of both luminal and mucosally adherent commensal bacteria. Each of these situations has been documented in CD patients (48).

The *NOD2* discovery also fulfilled the promise of genetics to identify mechanisms of IBD pathophysiology. Indeed, NOD2 kindled research into innate immunity as a major factor in CD pathophysiology. *NOD2* specificity for CD highlighted the importance of gut bacteria and complemented the association of antibodies against various gut microorganisms and effectiveness of antibiotic therapy for CD not UC. NOD2 mutations, however, were only involved in 25% of the CD population at risk in whites and were not present in Asians (49). The NOD2 association with CD was preceded by identification of well-replicated linkage in the pericentromeric region of chromosome 16 (50, 51).

HLA CLASS II ASSOCIATIONS

The HLA is the name of the major histocompatibility complex (MHC). This plays a key role in antigen presentation. MHC class 1 presents intra-cellular antigens to cytotoxic T cells. MHC class 2 present extra-cellular antigen, through antigen presenting cells, to T helper cells.

Both genetic linkage and association studies support the contribution of genetic variants within the major histocompatibility complex (MHC) region to IBD. The MHC region is characterized by enormous genetic diversity driven by significant selection forces, with complex patterns of linkage disequilibrium. However, several broad conclusions have been made. First, the strongest associations have been observed in the class II region as opposed to the class I region. Second, the associations between UC and CD are distinct, with evidence that class II associations are particularly strong for colonic disease. Replicated HLA class II associations in IBD include HLA-DRB1*1502 (serologic marker HLA-DR2) (52) association with UC and HLADRB1* 0103 association with UC and colonic CD (53, 54). HLA-DRB1*0103 is noteworthy in that it is a risk factor for both UC and colonic CD, suggesting that it may play a role in chronic inflammation of the colon independent of the major IBD phenotype (i.e., CD or UC) (54). As is the case with *IBD5*, the DRB1*0103 and DRB1*1502 class II variants are in strong linkage disequilibrium with SNPs on multiple immunologically active candidate genes. Whether the association signal is driven by class II genes themselves, in nearby genes, or in a combination of variants has not been fully defined. The advent of new genotyping platforms, including dense typing in this region, will result in a more

complete understanding of IBD associations in this region. However, HLA risk alleles in IBD are only a minor part of the genetic heritability puzzle, having at most a 3- to 4-fold risk (55).

ECM1

Among the loci not previously associated with CD, the strongest association with UC was found in *ECM1* on chromosome 1q21.2. *ECM1* is indeed an interesting molecule. It is expressed in the small and large intestine, interacts with the basal membrane–inhibiting metalloproteinase-9, and has been implicated in cell proliferation, angiogenesis, and cell differentiation (56). Lipoid proteinosis, also known as Urbach–Wiethe disease or hyalinosis cutis et mucosae, is a rare, mucocutaneous, autosomal-recessive disorder caused by *ECM1* mutations, resulting in a loss of function of this molecule (57). This condition is characterized by a marked thickening of the basement membrane in skin and mucosal surfaces, including the intestine. Such functional consequences of *ECM1* suggest a key role for *ECM1* in the structural organization of the dermis and maintenance of the epithelial function. Another potential link between *ECM1* and UC might be the capacity of *ECM1* of activating nuclear factor-B signaling (58), a well-known pathway implicated in the expression of many pro-inflammatory molecules (59). Finally, *ECM1* expression has been found up-regulated in several malignant epithelial tumors, including breast ductal carcinoma, esophageal squamous cell carcinoma, gastric cancer, and colorectal cancer. Of note, *ECM1* expression correlates with the metastatic properties of each of these tumors (60).

SLC22A4 AND *SLC22A5*

Two functional variants of the organic cation transporters OCTN1 and OCTN2 have been associated with CD in association with *CARD15* mutations (61). Mutations in the transcribed region of *SLC22A4*, which encodes OCTN1, and the promoter region of *SLC22A5*, which encodes OCTN2, affect the transcription and function of these carnitine and organic cation transporters. These variants are most actively expressed in the intestinal epithelium, macrophages and T cells, and cause decreased carnitine transport. Although many studies have associated the region of chromosome 5, which contains *SLC22A4* and *SLC22A5* with CD, some investigators are hesitant to identify the mutations in these genes as causative of CD because of the tight linkage disequilibrium that exists between multiple genes in this chromosomal region (61, 62).

THE *DLG5* GENE

Two haplotypes of *DLG5*, which encodes a scaffolding protein that helps to maintain epithelial integrity, have been associated with CD and combined UC and CD populations (63). Like the OCTN1 and OCTN2 variants, the 113G>A substitution in *DLG5* is associated with *CARD15* mutations in patients with CD. The association of the 113A *DLG5* allele with CD has been confirmed.

THE *MDR1* GENE

The multidrug resistance gene *MDR1* encodes P-glycoprotein 170, a transporter that governs efflux of drugs and possibly xenobiotic compounds from cells. P-glycoprotein 170 might also function as a 'flippase' that moves amphipathic substrates from the inner to the outer leaflet of the cell membrane. *MDR1* variants have been associated with UC (64) and CD (65). *MDR1* is of particular interest because it has been associated with treatment refractory IBD (66) and because mice in which *Mdr1* has been deleted develop colitis (56). Bone marrow transplantation studies have implicated epithelial and/or mesenchymal cells in the pathogenesis of colitis in

Mdr1-deficient mice. A study in Iran showed that the C3435T polymorphism of the MDR1 gene has an association with UC (67).

THE *VDR* GENE

Vitamin D has been known as a regulatory hormone of calcium homeostasis and bone mineralization, but other functions of vitamin D have included immunoregulatory effects. Many studies tried to clarify the role of vitamin D in immune system regulation, especially T cell-mediated immunization. Vitamin D suppresses lymphocyte proliferation and immunoglobulin synthesis. It inhibits the action of proinflammatory transcription factors, NF-κB, and the production of different cytokines including interleukin (IL)-2, IL-12, and interferon (INF)-δ. Moreover, Deluca and Cantorna showed that the activated form of vitamin D could limit the inflammatory process in mammalian cells. Undoubtedly, the vitamin D receptor is necessary for all known effects of the activated form of vitamin D. It is highly expressed on macrophages, monocytes, B and T lymphocytes and dendritic cells. VDR is a member of the steroid receptor family that mediates the effects of vitamin D by regulating transcription of multiple cellular genes. Several polymorphic sites were found on the VDR gene. Indeed, four polymorphisms recognized by restriction enzymes have been reported, including Apa I, Taq I and Bsm I at the 3′ flanking end of the VDR gene in exon 8 and Fok I at the 5′ end of this gene in exon 2. Previous studies revealed the association between VDR gene polymorphisms and the following disorders: prostate cancer, infectious diseases, type 1 diabetes mellitus, low bone mineral density in post menopausal women, malignant melanoma, chronic periodontitis, renal cell carcinoma, autoimmune hepatitis, breast cancer susceptibility and progression, Grave's disease, celiac disease, and IBD. It is noteworthy that in our study in Iran only a probable association of the Fok I polymorphism in VDR receptor gene and CD susceptibility was suggested. (68)

THE *PPARG* GENE

PPARG (peroxisome proliferative-activated receptor γ) variants have been linked to susceptibility in the SAMP1/YitFc mouse model of spontaneous chronic ileitis, and rare *PPARG* polymorphisms were found to be associated with human CD (69). PPARγ is a nuclear receptor that inhibits NFκB activity; its expression is decreased in patients with active UC (70), and its expression is upregulated by 5-aminosalicylic acid (71). In addition to a potential role in protecting against intestinal inflammation, treatment with the PPARγ ligand rosiglitazone was effective in an open-label trial involving UC patients (51), as well as in mouse experimental colitis (52).

THE INTERLEUKIN (IL) 23 RECEPTOR (*IL23R*) GENE

The first new genes discovered, interleukin (IL) 23 receptor (IL23R) having the next strongest risk (2- to 4-fold) for CD after NOD2 and the intestinal epithelial expressed autophagy gene, ATG16L1 (1.3–2.0-fold risk), identified consecutively in the US National Institute of Diabetes and Digestive and Kidney Diseases and the German non-synonymous screens, both delivered on the promise of IBD genetics. IL23R was also a risk factor, albeit of somewhat lower risk, for UC. Concurrent CD immunology and animal model research, and the therapeutic effectiveness of anti-p40, IL23/IL12 cytokine subunit antibodies showed that IL23 was an important cytokine for IBD chronic inflammation. The strong association with CD and UC put IL23 related genes and the Th17 pathway as a major focus of IBD research. The most highly associated allele, R281Q, has also been associated with

psoriasis. This genetic association may help explain the increased prevalence of IBD in psoriasis patients (4). Numerous IL23/TH17 pathway genes were associated, in addition to IL23R (72).

THE *IL-23* PATHWAY AND CHRONIC INFLAMMATION

The functional IL-23 receptor is heterodimeric, comprised of the *IL23R* subunit on chromosome 1p31, as well as *IL12RB1*, located on chromosome 19p13. The IL-23 cytokine is also heterodimeric and is composed of p19 (chromosome 12q13) and p40 (chromosome 5q33) subunits. The *IL12RB1* (receptor subunit) and p40 (cytokine subunit) are common to the IL-12 signaling pathway, with *IL12RB2* (chromosome 1p31) and p35 (chromosome 3q25) representing unique components of the functional IL-12 receptor and cytokine, respectively. Of interest, a genome wide association study (GWAS) analysed in a large UK cohort showed modest evidence for association near the *IL12B* (p40) subunit of the IL-23 cytokine (3,9). Interestingly, psoriasis cohorts show similar protective effects for Arg381Gln in *IL23R*, as is observed in IBD (73, 74). In contrast to the IBD findings, a strong association with psoriasis was observed for SNPs within the p40 gene region as well, potentially demonstrating overlapping and unique genetic features of chronic inflammatory disorders.

The human genetic associations with the IL-23 pathway coincide with significant advances in understanding of its role in mediating chronic inflammation. IL-23 has been recently associated with a new lineage of CD4 T cells, the Th17 lineage, which has now been linked to the pathogenesis of IBD (75-77). Th17 cells are distinct from classic Th1 and Th2 cells, both in the cytokines that induce their differentiation and the cytokines they produce. Th17 cell development is initiated from naive CD4 T cells by the sequential actions of the inductive cytokines, transforming growth factor, IL-6, and IL-23, which appear to act downstream of transforming growth factor IL-6–induced lineage commitment to amplify mature Th17 differentiation and the production of effector cytokines such as IL-17A, IL-17F, IL-21, and IL-22 (78-81). IL-21 was identified recently as an important intermediate in Th17 differentiation, which is induced by IL-6 and can act in an autocrine fashion to drive Th17 differentiation downstream of IL-6 (82-84).

While the shared component of the IL-12 and IL-23 receptors, IL-12R_1, is expressed by naive CD4 T cells, induction of the IL-23–specific component of the IL-23 receptor complex, IL-23R, is limited to developing Th17 cells downstream of IL-6 signaling via STAT3 (83, 85, 86).

Production of IL-23 appears limited to cells of the innate immune system, particularly dendritic cells and macrophages. The IL-23–specific component of IL-23, p19, is induced by stimulation of pattern recognition receptors, particularly TLRs and dectin receptors, which are activated by conserved structures of bacteria or fungi (87, 88). Recent studies indicate that IL-23 production is synergistically enhanced by coordinate stimulation of NOD family members. (89) Thus, bacterial or fungal components of the intestinal microbiota would be expected to activate IL-23 production by innate immune cells in the setting of an epithelial barrier breach and may continually stimulate low level IL-23 production in dendritic cells that sample the intestinal flora in the resting state (90). Although dysregulated Th1 responses to the microbiota have been traditionally associated with IBD, IL-23R is also expressed by mature dendritic cells and natural killer cells, where its function is less clearly defined but could also be important in disease pathogenesis. Accordingly, genetic variations that might enhance or blunt production of IL-23 by innate

immune cells or IL-23 signaling in adaptive T cells, natural killer cells, or dendritic cells might be expected to be significant genetic markers or risk factors for IBD. Similarly, additional factors associated with either the induction or function of the Th17 pathway might potentially be genetic susceptibility factors or protection for IBD. Suffice it to say that there are multiple genes and pathways associated with the development and function of Th17 cells that are likely to be elucidated as significant targets for analyses.

The genetic association and the pro-inflammatory role of IL-23 strongly prioritize this pathway as a therapeutic target in IBD. Anti-p40 administration, which blocks both IL-23 and IL-12 activities, has proven to be promising in the treatment of CD. The contribution of the *IL23R* pathway to IBD will likely involve more than simple gain or loss of function *IL23R* variants, and ongoing studies of this pathway may reveal new therapeutic options. Future studies should examine mechanisms of the strong protective effect of the Arg381Gln allele, which could potentially exploited for clinical benefit (91).

GENOME WIDE ASSOCIATION STUDIES IN IBD

In the last 2 years, GWAS of large cohorts of IBD patients have added new exciting information (18). Several GWAS studies have been very recently completed in CD patients, resulting in a large list of new CD-associated genes (6, 9, 92).

More importantly, these studies have clearly pointed toward three elements that are intimately involved in CD pathophysiology: recognition of microbial antigens, the IL-23/IL-17 pathway, and the mechanisms of autophagy. Each of them is, at present, the subject of intense investigation. The first merit of the work by Fisher et al. has been shifting the focus from CD to UC. Paralleling this evolution in genetics we have seen an evolution in biological therapy, new drugs assessed first in CD and then later, if successful, in UC patients have mirrored these genetic studies. The efficacy of these drugs, provides supporting clinical evidence ,of the the intricacy of UC genetics and the overlap existing between CD and UC with respect to risk factors conferring genetic susceptibility such as an exaggerated immune response, amenable to biological therapy (93). Multiple MHC markers show a strong association with UC. These findings imply that certain MHC-related pathogenic mechanisms are involved in the development of colonic IBD, regardless of the resulting type of intestinal inflammation. The study was also aimed at finding common genetic determinants for both CD and UC. Somewhat surprisingly, 5 of the 16 CD-associated loci identified in the published GWAS were also found to be a susceptibility locus for UC (94).

Progress from the Human Genome Project and HapMap Project, combined with markedly decreasing genotyping costs, has made possible the performance of adequately powered GWA studies in complex genetic disorders such as IBD. The first CD GWA success was the discovery in 2005 of variations of the tumor necrosis factor superfamily gene 15 (TNFSF15), using a low density GWAS platform of only 90,000 SNPs, as the first proven gene for Asian CD and a slightly lower risk gene for European CD (16). TNFSF15 can induce nuclear factor kappa-B and secretion of interferon gamma in T-cells that express death domain receptor 3 (DR3) (95). Importantly, recent reported associations in European ancestry CD cohorts identified through GWA studies have not been replicated in Asian cohorts**.** Greater comparative analyses of Asian and African IBD populations may provide important insights into alternative pathophysiologic mechanisms in IBD. Future genetic studies will be directed toward combining large GWAS data sets to identify common variation of more modest effects contributing to CD pathogenesis. Additional genetic, bioinformatic, and laboratory assessments

of association signals will be required to define susceptibility genes and contributing susceptibility alleles more clearly, especially where the association signals span several candidate genes. Similar GWAS studies in UC will provide important comparative insights to the CD GWA studies. The capacity of GWAS studies to sample common variation should be supplemented by deep resequencing efforts to identify rare variants contributing to IBD pathophysiology. Finally, successful translation of genetic advances to clinical practice will require improved understanding of intermediate phenotypes or biomarkers that can be objectively applied to measure disease activity, pathophysiologic mechanisms, and/or therapeutic response (96).

CONCLUSION

Novel findings on the genetics of IBD have fundamentally advanced understanding of the disease pathogenesis. A unique feature of the intestinal immune system is its close opposition to high concentrations of luminal bacteria. The initial association of the *NOD2* gene clearly established that altered host response in intracellular bacterial processing contributes to CD pathogenesis. This theme has been advanced with the report of CD associations with the *ATG16L1* gene and *IRGM* gene region, both of which are involved in intracellular bacterial processing and autophagy. For both *NOD2* and *ATG16L1*, the association appears to be unique to CD. A second major theme revolves around the importance of the IL-23 pathway in IBD pathogenesis, notably the presence of multiple associations within the *IL23R* gene to CD and UC. More modest associations of the *IL12B* and *PTPN2* genes implicate multiple members of the IL-23 pathway in IBD pathogenesis. The importance of the IL-23 pathway in mediating peripheral tissue inflammation generally is underscored by similar *IL23R* and *IL12B* associations observed in psoriasis as well as the association of *PTPN2* with type 1 diabetes (3, 73, 74).

Finally, the presence of CD and UC associations with potentially different patterns of associations in the *IL23R* and MHC regions may highlight important disease-modifying regions. The patterns of association in these two regions may provide important comparative insight between CD and UC.

Before GWAS studies, the *NOD2*, *IBD5*, and HLA class II associations represented the most consistently replicated associations. To date, four genes have been associated with CD and one with UC, and these data have been replicated. Strong associations with other chromosomal regions and genes (e.g., NFκB1, TLR5) have yet to be replicated, but such associations make it highly likely that many additional genes will be implicated in the pathogenesis of IBD, while others will be associated with extraintestinal disease (e.g., HLA-B27 and HLA-DR0103 human leukocyte antigen haplotypes, etc.) and with responses to pharmacologic treatment (i.e., pharmaco-genomics). The genes associated with the pathogenesis of IBD regulate innate immune responses, mucosal barrier function and bacterial killing. In addition, assessment for interactions between the multiple IBD associated genes will be important. Finally, genotype–phenotype correlation studies should help clinicians predict disease outcomes, including risk for complications, need for surgery, and response to therapy. Defining the genetic basis of IBD will be a major step for determining those at greatest risk of developing IBD and will uncover pathophysiological mechanisms to apply focused interventions for prevention and treatment. It may help predict disease course and complications and clarify the underlying nature of IBD type unclassified or "indeterminate colitis."

Although the genetic basis for CD has been outlined and relevance to UC is now being elucidated, the genetic basis for IBD requires further

investigation. However, how far do we have to go to complete the genetic portrait of IBD? Could discovery of IBD genes clarify the puzzle of IBD phenotype? Additional studies will be required to establish a definitive genetics role in IBD. Efforts to identify IBD susceptibility alleles before the advent of GWA studies involved focused candidate gene studies combined with fine-mapping efforts in regions of genetic linkage (demonstrations of increased genomic sharing between multiple, close relatives sharing a disease). The candidate gene approach resulted in well-replicated associations of IBD with various MHC class II associations. Such advancements could, in turn, lead to new and highly specific medical therapies that can target specific biological pathways based on better pathophysiologic understanding (4).

REFERENCES

1. Satsangi J, Silverberg MS, Vermeire S, Colombel JF. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 2006; 55: 749-53.

2. Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. Gastroenterology 2004; 126: 1504–17.

3. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447: 661-78.

4. Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet 2008; 40: 955- 62.

5. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, et al. A genome-wide association study identifies IL23r as an inflammatory bowel disease gene. Science 2006; 314: 1461-63.

6. Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, et al A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet 2007; 39: 207-11.

7. Kugathasan S, Baldassano RN, Brad- field JP, Sleiman PM, Imielinski M, Guthery SL, et al. Loci on 20q13 and 21q22 are associated with pediatric-onset inflammatory bowel disease. Nat Genet 2008; 40: 1211-15.

8. Libioulle C, Louis E, Hansoul S, Sandor C, Farnir F, Franchimont D, et al. Novel Crohn disease locus identified by genome-wide association maps to a gene desert on 5p13.1 and modulates expression of PTGER4. PLoS Genet 2007; 3: e58.

9. Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat Genet 2007; 39: 830-32.

10. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 2007; 39: 596-604.

11. Yamazaki K, McGovern D, Ragoussis J, Paolucci M, Butler H, Jewell D, et al Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. Hum Mol Genet 2005; 14: 3499-506.

12. McCarroll SA, Huett A, Kuballa P, Chilewski SD, Landry A, Goyette P, et al. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. Nat Genet 2008; 40: 1107-12.

13. Franke A, Balschun T, Karlsen TH , Sventoraityte J, Nikolaus S, Mayr G, et al Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. Nat Genet 2008; 40: 1319-23.

14. Franke A, Balschun T, Karlsen TH, Hedderich J, May S, Lu T, et al.. Replication of signals from recent studies of Crohn's disease identifies previously unknown disease loci for ulcerative colitis. Nat Genet 2008; 40: 713-15.

15. Targan SR, Karp LC. Defects in mucosal immunity leading to ulcerative colitis. Immunol Rev 2005; 206: 296–305.

16. Beckwith J, Cong Y, Sundberg JP, Elson CO, Leiter EH. Cdcs1, a major colitogenic locus in mice, regulates innate and adaptive immune response to enteric bacterial antigens. Gastroenterology 2005; 129:1473–1484.

17. Elson CO, Cong Y, McCracken VJ, Dimmitt RA, Lorenz RG, Weaver CT. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunol Rev 2005; 206: 260–76.

18. Cho JH. The genetics and immunopathogenesis of inflammatory bowel disease. Nat Rev Immunol 2008; 8: 458-66.

19. Russell RK, Satsangi J. IBD: a family affair. Best Pract Res Clin Gastroenterol 2004; 18: 525-39.

20. Orholm M, Iselius L, Sorensen TI, Munkholm P, Langholz E, Binder V.. Investigation of inheritance of chronic inflammatory bowel diseases by complex segregation analysis. BMJ 1993; 306: 20–24.

21. Brant SR, Shugart YY. Inflammatory bowel disease gene hunting by linkage analysis: rationale, methodology, and present status of the field. Inflamm Bowel Dis 2004; 10: 300–11.

22. Anderson CA, Massey DCO, Barrett JC, Prescott NJ, Tremelling M, Fisher SA, Gwilliam R, et al. Investigation of Crohn's disease risk loci in ulcerative colitis further defines their molecular relationship. Gastroenterology 2009; 136: 523–29.

23. Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, et al. NOD2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem 2003; 278: 8869–72.

24. Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem 2003; 278: 5509–12.

25. Bonen DK, Ogura Y, Nicolae DL, Inohara N, Saab L, Tanabe T, et al. Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. Gastroenterology 2003; 124: 140–46.

26. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 2001; 411: 599–603.

27. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 2001; 411: 603–606.

28. Lesage S, Zouali H, Cezard JP, Colombel JF, Belaiche J, Almer S, Sargent M, Datta LW, Nouvet F, et al. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. Am J Hum Genet 2002; 70: 845–57.

29. Economou M, Trikalinos TA, Loizou KT, Tsianos EV, Ioannidis JP. Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a metaanalysis. Am J Gastroenterol 2004; 99: 2393–404.

30. Brant SR, Wang MH, Rawsthorne P, Sargent M, Datta LW, Nouvet F, et al. A population-based case-control study of CARD15 and other risk factors in Crohn's disease and ulcerative colitis. Am J Gastroenterol 2007; 102: 313–23.

31. Hugot JP, Zaccaria I, Cavanaugh J, Yang H, Vermeire S, Lappalainen M, et al. Prevalence of CARD15/NOD2 mutations in Caucasian healthy people. Am J Gastroenterol 2007;102:1259–1267.

32. Chamaillard M, Philpott D, Girardin SE, Zouali H, Lesage S, Chareyre F, et al. Gene-environment interaction modulated by allelic heterogeneity in inflammatory diseases. Proc Natl Acad Sci U S A 2003;100:3455–3460.

33. Leong RW, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, et al. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. Aliment Pharmacol Ther 2003; 17: 1465–70.

34. Newman B, Siminovitch KA. Recent advances in the genetics of inflammatory bowel disease. Curr Opin Gastroenterol 2005 21: 401–407

35. Derakhshan F, Naderi N, Farnood A, Firouzi F, Habibi M, Rezvany MR, et al. Frequency of three common mutations of CARD15/NOD2 gene in Iranian IBD patients. Indian J Gastroenterol 2008; 27: 8-11.

36. ENCODE Project Consortium. The ENCODE (ENCyclopedia Of DNA Elements) Project. Science 2004; 306: 636–40.

37. Maeda S, Hsu LC, Liu H, Bankston LA, Iimura M, Kagnoff MF, et al. NOD2 mutation in Crohn's disease potentiates NF-kappaB activity and IL-1beta processing. Science 2005; 307: 734–38.

38. Kobayashi KS, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nuñez G, et al. NOD2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 2005; 307: 731–34.

39. Pauleau AL, Murray PJ. Role of NOD2 in the response of macrophages to toll-like receptor agonists. Mol Cell Biol 2003; 23: 7531–539.

40. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol 2004; 5: 800–808.

41. Watanabe T, Kitani A, Murray PJ, Wakatsuki Y, Fuss IJ, Strober W. Nucleotide binding oligomerization domain 2 deficiency leads to

dysregulated TLR2 signaling and induction of antigen-specific colitis. Immunity 2006; 25: 473–85.

42. Hisamatsu T, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, Podolsky DK. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology 2003; 124: 993–1000.

43. Wehkamp J, Harder J, Weichenthal M, Schwab M, Schäffeler E, Schlee M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. Gut 2004; 53: 1658–64.

44. Abraham C, Cho JH. Functional consequences of NOD2 (CARD15) mutations. Inflamm Bowel Dis 2006; 12: 641–50.

45. Lala S, Ogura Y, Osborne C, Hor SY, Bromfield A, Davies S, et al. Crohn's disease and the NOD2 gene: a role for Paneth cells. Gastroenterology 2003; 125: 47–57.

46. Wehkamp J, Salzman NH, Porter E, Nuding S, Weichenthal M, Petras RE, et al. Reduced Paneth cell α-defensins in ileal Crohn's disease. Proc Natl Acad Sci USA 2005; 102: 18129–34.

47. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell 2004; 118: 229–41.

48. Swidsinski A, Ladhoff A, Pernthaler A, Swidsinski S, Loening-Baucke V, Ortner M, et al. Mucosal flora in inflammatory bowel disease. Gastroenterology 2002; 122: 44–54.

49. Schreiber S, Rosenstiel P, Albrecht M, Hampe J, Krawczak M. Genetics of Crohn disease, an archetypal inflammatory barrier disease. Nat Rev Genet 2005; 6: 376–388

50. Lewis JD, Lichtenstein GR, Stein RB, Deren JJ, Judge TA, Fogt F, et al. An open-label trial of the PPAR-γ ligand rosiglitazone for active ulcerative colitis. Am J Gastroenterol 2001; 96: 3323–28.

51. Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, et al. A novel therapy for colitis utilizing PPAR-γ ligands to inhibit the epithelial inflammatory response. J Clin Invest 1999; 104: 383–89

52. Yang H, Rotter JI, Toyoda H, Landers C, Tyran D, McElree CK, Targan SR. Ulcerative colitis: a genetically heterogeneous disorder defined by genetic (HLA class II) and subclinical (antineutrophil cytoplasmic antibodies) markers. J Clin Invest 1993; 92: 1080–84.

53. Trachtenberg EA, Yang H, Hayes E, Vinson M, Lin C, Targan SR, et al. HLA class II haplotype associations with inflammatory bowel disease in Jewish (Ashkenazi) and non-Jewish caucasian populations. Hum Immunol 2000; 61: 326–33.

54. Silverberg MS, Mirea L, Bull SB, Murphy JE, Steinhart AH, Greenberg GR, et al. A population- and family-based study of Canadian families reveals association of HLA DRB1*0103 with colonic involvement in inflammatory bowel disease. Inflamm Bowel Dis 2003; 9: 1–9.

55. Panwala CM, Jones JC, Viney JL. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, mdr1a, spontaneously develop colitis. J Immunol 1998; 161: 5733–44.

56. Sercu S, Zhang L, Merregaert J. The extracellular matrix protein 1: its molecular interaction and implication in tumor progression. Cancer Invest. 2008; 26: 375-84.

57. Chan I, Liu L, Hamada T, Sethuraman G, McGrath JA. The molecular basis of lipoid proteinosis: mutations in extracellular matrix protein 1. Exp Dermatol 2007; 16: 881–90.

58. Matsuda A, Suzuki Y, Honda G, Muramatsu S, Matsuzaki O, Nagano Y, et al. Large-scale identification and characterization of human genes that activate NF-kappaB and MAPK signaling pathways. Oncogene 2003; 22: 3307-18.

59. Maloy KJ. The Interleukin-23 / Interleukin-17 axis in intestinal inflammation. J Intern Med 2008; 263: 584-90.

60. Wang L, Yu J, Ni J, Xu XM, Wang J, Ning H, et al. Extracellular matrix protein 1 (ECM1) is over-expressed in malignant epithelial tumors. Cancer Lett 2003; 200: 57-67.

61. Peltekova VD, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, et al. Functional variants of OCTN cation transporter genes are associated with Crohn disease. Nat Genet 2004; 36: 471–75.

62. Noble CL, Nimmo ER, Drummond H, Ho GT, Tenesa A, Smith L, et al. The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease. Gastroenterology. 2005; 129: 1854-64.

63. Stoll M, Corneliussen B, Costello CM, Waetzig GH, Mellgard B, Koch WA, et al. Genetic variation in DLG5 is associated with inflammatory bowel disease. Nat Genet 2004; 36: 476–80.

64. Ho GT, Nimmo ER, Tenesa A, Fennell J, Drummond H, Mowat C, et al. Allelic variations of the

multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. Gastroenterology 2005; 128: 288–96.

65. Brant SR, Panhuysen CI, Nicolae D, Reddy DM, Bonen DK, Karaliukas R, et al. MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. Am J Hum Genet 2003; 73: 1282–92.

66. Farrell RJ, Murphy A, Long A, Donnelly S, Cherikuri A, O'Toole D, et al. High multidrug resistance (P-glycoprotein 170) expression in inflammatory bowel disease patients who fail medical therapy. Gastroenterology 2000; 118: 279–88.

67. Farnood A, Naderi N, Moghaddam SJ, Noorinayer B, Firouzi F, Aghazadeh R, et al. The frequency of C3435T MDR1 gene polymorphism in Iranian patients with ulcerative colitis. Int J Colorectal Dis 2007; 22: 999-1003.

68. Naderi N, Farnood A, Habibi M, Derakhshan F, Balaii H, Motahari Z, et al. Association of vitamin D receptor gene polymorphisms in Iranian patients with inflammatory bowel disease. J Gastroenterol Hepatol 2008; 23: 1816-22.

69. Sugawara K, Olson TS, Moskaluk CA, Stevens BK, Hoang S, Kozaiwa K, et al. Linkage to peroxisome proliferator-activated receptor-γ in SAMP1/YitFc mice and in human Crohn's disease. Gastroenterology 2005; 128: 351–60.

70. Dubuquoy L, Jansson EA, Deeb S, Rakotobe S, Karoui M, Colombel JF, et al. Impaired expression of peroxisome proliferator-activated receptor γ in ulcerative colitis. Gastroenterology 2003; 124: 1265–76.

71. Rousseaux C, Lefebvre B, Dubuquoy L, Lefebvre P, Romano O, Auwerx J, et al. Intestinal anti-inflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-γ. J Exp Med 2005; 201: 1205–15

72. Cho JH, Weaver CT. The genetics of inflammatory bowel disease. Gastroenterology 2007; 133: 1327–39.

73. Capon F, Di Meglio P, Szaub J, Prescott NJ, Dunster C, Baumber L, et al. Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. Hum Genet 2007; 122: 201–206.

74. Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. Am J Hum Genet 2007; 80: 273–90.

75. Elson CO, Cong Y, Weaver CT, Schoeb TR, McClanahan TK, Fick RB, et al. Monoclonal anti-interleukin 23 reverses active colitis in a T cell-mediated model in mice. Gastroenterology 2007; 132: 2359–70.

76. Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 2007; 25: 821–52.

77. Yen D, Cheung J, Scheerens H, Poulet F, McClanahan T, McKenzie B, et al. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. J Clin Invest 2006; 116: 1310–16.

78. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006; c441: c235–38.

79. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature 2006; 441: 231–34.

80. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 2006; 24: 179–89.

81. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, et al. Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature 2007; 445: 648–51.

82. Korn T, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature 2007; 448: 484–87.

83. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature 2007; 448: 480–83.

84. Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol 2007; 8: 967–74.

85. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4_ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005; 6: 1123–32.

86. Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS, et al. STAT3 regulates

cytokine-mediated generation of inflammatory helper T cells. J Biol Chem 2007; 282: 9358–63.

87. Leibundgut-Landmann S, Gross O, Robinson MJ, Osorio F, Slack EC,et al. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat Immunol 2007; 8: 630–38.

88. Napolitani G, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. Nat Immunol 2005; 6: 769–76.

89. Fritz JH, Le Bourhis L, Sellge G, Magalhaes JG, Fsihi H, Kufer TA, et al. NOD1-mediated innate immune recognition of peptidoglycan contributes to the onset of adaptive immunity. Immunity 2007; 26: 445–59.

90. Becker C, Wirtz S, Blessing M, Pirhonen J, Strand D, Bechthold O, et al. Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. J Clin Invest 2003; 112: 693–706.

91. Mannon PJ, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, et al. Anti-interleukin-12 antibody for active Crohn's disease. N Engl J Med 2004; 351: 2069– 79.

92. Prescott NJ, Fisher SA, Franke A, Hampe J, Onnie CM, Soars D, et al. A nonsynonymous SNP in ATG16L1 predisposes to ileal Crohn's disease and is independent of CARD15 and IBD5. Gastroenterology 2007; 132: 1665-71.

93. Stokkers PC, Reitsma PH, Tytgat GN, van Deventer SJ. HLA-DR and -DQ phenotypes in inflammatory bowel disease: a meta-analysis. Gut 1999; 45: 395-401.

94. Sans M, Castells A. Ulcerative colitis and Crohn's disease genetics: more similar than we thought? Gastroenterology 2008; 135: 1796-98.

95. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature 2001; 409: 860–21.

96. Yamazaki K, Onouchi Y, Takazoe M, Kubo M, Nakamura Y, Hata A. Association analysis of genetic variants in IL23R, ATG16L1 and 5p13.1 loci with Crohn's disease in Japanese patients. J Hum Genet 2007; 52: 575–83.