The genetics of inflammatory bowel disease: diagnostic and therapeutic implications

Carmen Cuffari
Baltimore, USA

Background: **The genetics of inflammatory bowel diseases (IBD) has brought new insight into the spectrum of disease phenotypes that are collectively labeled as either Crohn's disease or ulcerative colitis. In concert with the pharmacogenomics of drug therapy, it has led clinicians to develop the notion of a more tailored approach to therapy.**

Data sources: **Articles were searched from PubMed (1995-2010) with key words "inflammatory bowel diseases", "Genetics", "pharmacogenomics".**

Results: **Among all the putative susceptibility loci, the *NOD2* gene has been the most studied and linked to an aggressive form of stricturing and perforating disease of the ileum. Other potential gene polymorphisms, including those encoding for the interleukin-23 receptor, have lent themselves to the recent development of potential novel immunosuppressive therapies. While the linkage of a number of autophagy genes with either Crohn's disease or ulcerative colitis has provided insight into the innate adaptive immune pathway's response to commensual intestinal bacteria. Pharmacogenetic polymorphisms of azathioprine metabolism have been shown to predict toxicity to anti-metabolite therapy. Patients with absent thiopurine methyl transferase enzyme activity are at risk for irreversible bone marrow suppression, and are not considered good candidates for either 6-mercaptopurine (6-MP) or azathioprine therapy.**

Conclusions: **Ultimately, the correlation between these genotypes and clinical phenotype of disease will inevitably lead to an improved understanding of disease natural history and a more tailored approach to therapy. Although there is ongoing debate as to whether these inherent differences in enzyme activity can predict responsiveness to anti-metabolite therapy, some gastroenterologists do find value in 6-MP metabolite testing as a means of monitoring patient compliance and tailoring the dose of anti-metabolite therapy based on a perceived therapeutic window. In the future, patients with IBD will ultimately be categorized based on their genomic imprint to allow for a better delineation of disease phenotype. Furthermore, the application pharmacogenomics of drug therapy into clinical practice will be pivotal in maximizing treatment response while avoiding untoward side-effects.**

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Author Affiliations: Division of Pediatric Gastroenterology, The Johns Hopkins University School of Medicine, 600N. Wolfe St. Brady 320, Baltimore, MD, USA 21287 (Cuffari C)

Corresponding Author: Carmen Cuffari, MD, Associate Professor of Pediatrics, Division of Pediatric Gastroenterology, The Johns Hopkins University School of Medicine, 600N. Wolfe St. Brady 320, Baltimore, MD, USA 21287 (Email: ccuffari@jhmi.edu)

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Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are chronic, idiopathic inflammatory bowel disorders that are commonly diagnosed in the pediatric age group.[1] The majority of pediatric cases are diagnosed between 10-16 years of age, and account for 25% of all cases of inflammatory bowel diseases (IBD).[2] At the Johns Hopkins Children's Center, more than 600 children and adolescents with IBD are actively followed. Among these, approximately 25% have UC. Over the last several years we have noticed a three-fold increase in new cases of CD, whereas the incidence of UC has remained stable.

Although a number of critical pathways have been defined in the immune pathogenesis of IBD, the lines of evidence suggesting genetic factors as a component of susceptibility have been extensively documented. Indeed, a positive family history is probably the most important risk factor for developing IBD.[3,4] It is well recognized that cases of CD cluster within families. The concordance for the occurrence of CD in monozygotic twins is higher than in fraternal twins, indicating that genetics may account in part for some of this familial clustering.[5] The fact that the concordance of CD in monozygotic twins is not absolute indicates that genes alone are not sufficient to produce disease. Indeed, a complex interaction between environmental triggers

in a genetically predisposed individual is believed to be required for disease expression. The focus of this review is to examine some of the recent advances in our understanding of the pathogenesis of IBD, and highlight several innovative treatment strategies based on pharmacogenomic principles.

Genetic factors

The lifetime risk of developing UC and CD in the first degree relatives of a proband is 1.6% and 5.2%, respectively. When the proband is of Askenazi Jewish descent, the risk of developing CD in offspring increases to 10%-12.6%.[4] Other examples pointing toward the importance of genetic factors include: the higher risk of IBD in children when both parents are affected, ethnic variations, and the association with several genetically determined genetic diseases including ankylosing spondylitis[6] and Turner syndrome.[7] Family studies have also documented the occurrence of both CD and UC in the same family, thereby suggesting that these two diseases may share either one or more genes in common.[8] Unlike CD, the concordance for UC in monzygotic twins is significantly lower (<20%), thereby reducing the theoretical probability of identifying a single UC susceptibility locus, and raises the notion of genetic pleiotropism.

The genetic pattern observed in families with several affected members with IBD does not adhere to a simple Mendelian model of inheritance. Furthermore, when one assesses these multiplex families, 25% are mixed, one member with CD, and the other with UC. This pattern of inheritance would suggest that specific susceptibility genes are likely shared, and that a multi-genetic inheritance is probably required in order to develop intestinal disease. Genome wide searches for susceptibility loci in CD and UC have been pursued in several countries. A linkage area on chromosome 16 (IBD1 locus) has been associated with a relative risk of 1.3 in acquiring CD that is equivalent to the relative risk assigned to families with a tendency toward diabetes.[9] This linkage on chromosome 16 has now been confirmed in at least 5 other study groups, 3 in the United States, 1 in Australia and 1 in Germany.[10-14] The strongest linkage for the IBD1 susceptibility locus on chromosome 16 was identified in affected family members with an age at disease onset before age 22. At least one family member from each of these 57 pedigrees studied had aggressive CD that required either surgical intervention or immunomodulatory therapy. This study was the first to suggest that the causative gene for CD may be linked to a specific clinical phenotype.[15] Interestingly, there are several candidate genes on Chromosome 16 that have also been considered in the pathogenesis of IBD, including CD19β-lymphocyte genes, IL-4 and CDII integrin cluster.[16,17]

The gene known as *NOD-2* (nucleotide oligomerization domain 2) on chromosome 16 has been closely linked in several multiplex families with CD.[18,19] *NOD-2* is expressed in peripheral mononuclear cells, and is involved in NFκB activation, a key transcription factor in the activation of genes that mediate the inflammatory cascade. The NOD-2 protein contains a caspase recruitment domain (*CARD*) that induces apoptosis, an important mechanism in immune inactivation, and a leucine rich domain (*LRR*) involved in the interaction with infecting bacterial lipopolysaccharides. On exposure to bacterial lipopolysaccharides, LRR induces NFκB activation (Fig. 1).[20] Different mutations in the *NOD-2* gene have been recognized in the leucine rich domain, the most important include: Leu1007fsinsC, Arg702Trp, and Gly908Arg. All of these mutations have been associated in some multiplex families with CD. How these mutations are perceived to facilitate the onset of CD is not well understood. It is believed that these mutations either perturb the homeostatic relationship between the intestinal flora and the gut associated lymphoid tissue or lead to an exaggerated innate immune response to antigenic stimulation in affected patients with CD.[20]

Phenotype genotype correlation studies would suggest that *NOD-2* mutations are likely to be associated in patients with ileal CD of early onset.[19] The same association has not been identified in the Japanese population with CD. Indeed, mutations in *NOD-2* have not been observed in the Japanese IBD patient population. Future studies may allow us to identify specific aberrant gene alleles, including *NOD-2* that can be used to define an individual's likelihood of developing IBD and possibly predict responsiveness to therapy.

Single nucleotide polymorphisms (SNPs) within the *CARD15* gene (1007fs) were associated with a seventy-fold increased risk for developing a stricturing phenotype.[21] In mice lacking the *CARD15*/*NOD2* genotype, there was a decreased number of Paneth cells similar to that seen in patients with CD homozygous for *CARD15*/*NOD2* mutations.[22] Paneth cells provide a gastrointestinal barrier against microbes in the small intestines. Diminution may imply impaired bacterial

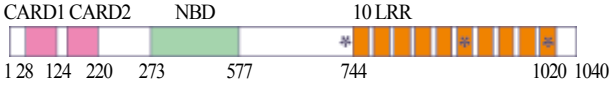

Fig. 1. Nucleotide oligomerization domain 2 (NOD-2) protein. LRR: leucine-rich repeats; CARD: caspase recruitment domain; NBD: nucleotide binding domain.

sensing as a major contributor to intestinal inflammation or an abnormal activation of the immune system in response to normal commensual bacteria.[23]

In addition to the *NOD2/CARD15* gene, two novel polymorphisms in the solute carrier family (*SLC22A4/A5*) within the *IBD5* gene have been identified in a Canadian patient. These two CD-associated alleles encode the organic cation transporters 1 and 2, respectively; both associated with early onset CD. However, the effect is modest and lower compared to adult-onset CD.[24]

Genome-wide associations have now defined more than 30 potential susceptibility loci for CD; some will be described herein. Another gene of interest is the *DLG5* gene located on chromosome 10 that encodes for a scaffolding protein involved in maintaining normal epithelial death or growth. A total of three IBD-associated genetic variations in the *DLG5* gene have been found. Multivariate logistic regression analysis has shown just a modest susceptibility factor among several large cohorts of patients with IBD.[25]

Toll-like receptors (TLRs) are single membrane-spanning receptors providing an innate immune response to gastrointestinal bacteria that have breached the mucosa. Moreover, they are also able to distinguish pathogenic from normal commensal bacteria.[26] It has been observed that *TLR4* is over expressed in the epithelial cells isolated from patients with CD, *TLR3* is down regulated, while *TLR2* and *TLR5* remain unchanged.[27] Despite the protective role of certain TLRs at the intestinal mucosal interface, only minor genetic variations affecting the TLR signaling pathway have been identified in patients with IBD.[28,29]

Duerr and coworkers[30] found a highly significant variation in the gene encoding the interleukin-23 receptor (IL23-R) among patients with CD. IL-23 is a cytokine comprised two different subunits, one called p40 sub-unit, which is also shared with the cytokine IL-12. IL-23 is an important part of the early inflammatory response against bacteria. This specific gene has also been correlated with the inflammatory cytokines generated by IL-12. IL-12 transforms CD_4^+ T lymphocytes into a Th1 immune pathway, and in the company of the IL-23 lead into the Th17 immune pathway. The Th17 pathway is characterized by the production of other inflammatory agents IL-17, TNF, and IL-6.[31] All these cytokines are expressed in several autoimmune inflammatory responses including arthritis and chronic intestinal inflammation. Various bacterial components including peptidoglycan are found to exercise a regulatory effect on the antigen presenting cells, thereby increasing IL-23 expression without affecting IL-12. This further illustrates how crucial even the smallest variation in IL-12 and IL-23 dependent inflammatory mechanisms may be on the impact of a specific inflammatory phenotype.[32]

Authophagy is the recycling behavior of cellular organelles. Furthermore, it provides the normal defense against microorganisms that have migrated into the cytoplasm of the cells. In some cases, autophagy has also been shown to be involved in the binding of an antigen into a MHC binding groove within the cell surface. Due to their role in the recognition and destruction of antigens, it has been reasonable to speculate a potential role in the IBD inflammatory pathway.[33] The autophagy gene *ATG16L1* has been identified on chromosome 2q37. The signal of this gene is identified as coding for a SNP changing the amino-acid sequence coding for a threonine to alanine substitution. The *ATG16L1* protein is a key component in autophagy, and has been shown to be expressed in CD4 lymphocytes within the gastrointestinal tract.[34]

The *IRGM* gene is also involved in autophagy. A highly significant association was found between variation in the *IRGM* gene and susceptibility to CD.[35] Mice without the gene have an impaired ability to destroy the pathogens Toxoplasma gondii and Listeria monocytogenes. Human macrophages with a knockdown of the *IRGM* gene allow the prolonged survival of the mycobacteria tuberculosis.[36]

The data for *ATG16L1* and *IRGM* clearly implicates defects in the mechanisms of autophagy in the pathogenesis of CD.[37] These findings may also explain the presence of sub-pathogenic organisms such as adherent invasive *Escherichia coli* within intestinal epithelial cells in patients with CD. Defects in autophagy might provide an answer as to whether the intestinal inflammation identified among patients with IBD is either a consequence of an inappropriate clearance of normal commensual bacteria or failure to regulate the adaptive immune pathway response.

Congenital immune deficiency syndromes

Several primary immunodeficiency syndromes have been associated with an IBD like clinical and histopathological presentations.[38] Patients may present with clinical signs and symptoms indistinguishable from those with IBD, including chronic abdominal pain, diarrhea and colitis like symptoms. Indeed, extra-intestinal manifestations like failure to thrive, developmental delay and perianal diseases are common presenting complaints.

Common variable immunodeficiency is a rare disorder that is characterized by variable alterations in T and B lymphocyte cell function. Patients with a predominant humoral defect may present with nonspecific enteritis or enterocolitis that may improve

with antibiotic or immunoglobulin therapy. Whereas, those patients with a predominantly cellular defect may present with an IBD like intestinal disorder that may respond to corticosteroid therapy.[39] One such patient treated at the Johns Hopkins Children Hospital responded well to an oral 5-ASA preparation in combination with low dose corticosteroid therapy.

Leukocyte adhesion deficiency is an immune deficiency disorder. The patients with this disorder have a deficiency in several specific plasma membrane glycoproteins that play a role in lymphocyte adhesion and cellular toxicity. The radiological findings of the terminal ileum may be indistinguishable from those in patients with Crohn's ileocolitis.[40] One such patient treated at our Children's Center with CD-like enterocolitis failed in medication management and ultimately required a bone marrow transplant.

Other such immunodeficiency states including chronic granulomatous disease, glycogen storage disease type 1b and Hermansky Pudlak syndrome (Table) have unique immune defects that present with symptoms indistinguishable from those of CD. Colonic biopsies will show multi-focal areas of chronic inflammation with multi nucleated giant cells.[40] The precise role of any of these specific immune defects in the pathogenesis of IBD is unclear.

Environmental factors

IBD is thought to be the result of an inter-play between one or more environmental factors in genetically susceptible individuals. Epidemiological studies have suggested that IBD is more common in industrialized nations, and is rare in the developing world.[40] Although a number of dietary factors including the increased consumption of refined sugars and polyunsaturated fats have been used as a possible explanation for these differences, no specific proof has been cited. Conversely, other environmental factors including poor sanitation and exposure to intestinal parasites could influence intestinal immunity, and reduce susceptibility in genetically predisposed individuals. The diagnosis of new onset CD in families who have recently immigrated to North America may in part be attributed to as yet undefined exposure to disease precipitating environmental antigens.[40]

Perinatal health events have been associated with an increased risk of developing IBD. Ekbom and coworkers[41] have suggested that prenatal maternal infection and pregnancies complicated by preeclampsia, threatened miscarriage, and gestational diabetes were potential risk factors. The same group has also suggested that perinatal exposure to the measles virus may play a role in the pathogenesis of CD.[42] In 4 women who had measles infections during pregnancy, 3 of their offsprings developed CD, while the fourth experienced measles infection at birth.[43] Transmission electron microscopy has been able to identify paramyxoviral-like particles and inclusion bodies within the giant cells in intestinal tissue biopsies from patients with CD.[44] Moreover, persistent measles infection has been detected in the intestinal tissue of 5 patients with CD by immunogold electron microscopy.[45] Epidemiological studies from Sweden would also suggest that there is an increased incidence of CD but not UC in people born during periods of documented measles epidemics.[46] These studies have raised the possibility that the neonate is more susceptible to either acute or persistent measles infection on account of its immature immune system. Although immune tolerance may explain the persistent measles infection in these patients, the pathogenesis of intestinal inflammation remains unclear. The lack of a negative effect of measles vaccination in lowering the incidence of CD, which in fact has shown a four-fold rise over the last decade, would go against the measles virus as playing an important role in IBD pathogenesis. The presence of measles viruses in patients with CD may be an epiphenomenon of limited importance; however, the role of attenuated measles virus vaccines in the development of CD remains unclear.

Table. Primary immunodeficiency disorders

T and B cell disorders
Common variable immune deficiency
Severe combined immune deficiency
Neutrophil disorders
Chronic granulomatous disease
Hermansky Pudlak syndrome
Leukocyte adhesion deficiency
Glycogen storage disease type 1b

The pharmacogenomics of immunosuppressive therapy

Immunomodulatory agents including azathioprine and 6-mercaptopurine (6-MP) have been increasingly utilized in steroid dependent IBD. Over the last 10 years, the knowledge base has grown so significantly with regard to the pharmacology of these agents that both consensus and debate have developed as to the influence of pharmacogenomics on patient responsiveness to anti-metabolite therapy and susceptibility to drug induced toxicity.

6-MP and its parent drug azathioprine are well known for their immunosuppressive and lymphocytotoxic properties. They act by interfering with protein synthesis and nucleic acid metabolism in the sequence that follows

antigen stimulation, as well as by their cytotoxic effects on lymphoid cells. This direct cytotoxicity against lymphoid cells is the basis for their use in the treatment of lymphoma and leukemias.[47] 6-MP undergoes rapid and extensive catabolic oxidation to 6-thiouric acid in the intestinal mucosa and liver by the enzyme xanthine oxidase. As proof, the absolute bioavailability of 6-MP ranges from 5% to 37%. The anabolic transformation of 6-MP into active ribonucleotide metabolites occurs intracellularly along the competing routes catalyzed by thiopurine methyl-transferase (TPMT) and hypoxanthine phosphoribosyl transferase (HPRT), giving rise to 6-methyl-mercaptopurine (6-MMP), 6-methyl- thioinosine 5'-monophosphate (6-Me-tiMP) and 6-thioguanine nucleotides (6-TG), respectively (Fig. 2).[48] The time to reach a steady-state for these active metabolites may take up to several months,[49] and may explain the delayed median clinical response time of 3-4 months in patients on 6-MP therapy.

An apparent genetic polymorphism has been observed in TPMT activity (Fig. 2). Negligible activities were noted in 0.3%, and low activities in 11% of individuals.[50] Indeed, the low TPMT activities have been associated with increased cytotoxicity, by allowing 6-MP metabolism to be shunted toward the excessive production of 6-TG nucleotides.[51] Studies in children with leukemia have shown that the risk of clinical relapse and absolute leukocyte count correlates inversely with erythrocyte 6-TG concentrations.[52]

The measurement of erythrocyte 6-MP metabolites 6-TG and 6-MMP has been proposed as a useful clinical tool for measuring clinical efficacy, documenting patient compliance to therapy and explaining some drug induced toxicity in patients with IBD. In our preliminary study in adolescent patients with CD on long-term 6-MP therapy, high performance liquid chromatography measurement of erythrocyte 6-TG metabolite levels showed an inverse correlation with disease activity. Although a wide range of metabolite levels was associated with a favorable clinical response, patients with high 6-TG levels (>250 pmol/8×10^8 RBCs) were uniformly asymptomatic.[52] Similar results have been reported in 93 pediatric and 45 adult patients with IBD in whom disease remission correlated well with erythrocyte 6-TG levels of more than 230 and 260 pmol/8×10^8 RBCs, respectively.[53,54] All of these studies support the putative immunosuppressive role of 6-TG in patients with IBD. Prospective studies in 15 pediatric and 22 adult patients with steroid dependent CD showed that treatment efficacy correlated with improved erythrocyte 6-TG metabolite levels following an adjustment in anti-metabolite therapy.[55,56] All patients were initially described as unresponsive to anti-metabolite therapy despite receiving therapeutic drug dosages for at least 12 weeks. Interestingly, clinical remission was achieved by optimizing the dose of either azathioprine or 6-MP without inducing leukopenia. These studies would suggest that the measurement of erythrocyte 6-TG levels can be used to optimize the dose of anti-metabolite therapy to achieve a desired therapeutic effect while avoiding drug induced toxicity. Indeed, patients who remain symptomatic despite therapeutic 6-TG levels should be considered for other forms of medical or surgical therapy.

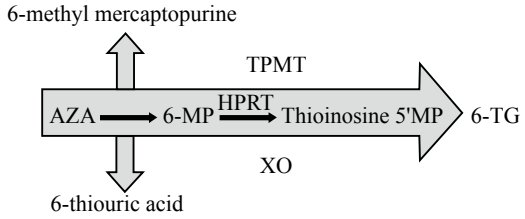

Fig. 2. 6-mercaptopurine metabolism: patient susceptibility to 6-MP induced toxicity is determined by pharmacogenomic differences in TPMT enzyme activity levels. AZA: azathioprine; 6-MP: 6-mercaptopurine; 6-TG: 6-thioguanine nucleotides; HPRT: hypoxanthine phosphoribosyl transferase; TPMT: thiopurine methyl-transferase.

Many pediatricians have been reluctant to prescribe 6-MP on account of potential drug related toxicity, including pancreatitis 3%, bone marrow depression 2%, super-infection 7%, and hepatitis 0.3%.[57] A large pediatric series reported similar frequencies of 6-MP related side-effects in patients with CD on long-term 6-MP therapy.[58] Severe side-effects are either idiosyncratic or related to genetic polymorphism as described above. Black and coworkers[59] suggested the notion that pharmacogenetic differences in 6-MP metabolism influence a patient's risk of drug toxicity. In that study, five of six adult patients with CD who experienced 6-MP related cytotoxicity had reduced TPMT enzyme activity levels.[59] Genetic polymorphisms may have a role to play in determining the long-term risk for malignancy. In several pediatric oncology studies, the risk for secondary malignancies, including acute myeloblastic leukemia and myelodysplasia was higher in children with low TPMT activity levels with acute lymphocytic leukemia on maintenance 6-MP therapy.[60] Larger longitudinal studies are necessary in order to draw a correlation between the pharmacogenomics of 6-MP metabolism and the long-term risk of malignancy in patients with IBD.

Conclusions

Recent advances in genetic testing have confirmed the presence of a susceptibility locus (*NOD-2*) on chromosome 16 in a sub-group of patients with CD. The

NOD-2 gene has shown a strong association with CD of the ileum, and may explain the genetic heterogeneity of IBD. Whether multiple genotypes will be found to correlate with the various disease phenotypes that are grouped with the diagnosis of CD remains to be determined. Pharmacogenomic differences in 6-MP metabolism predict susceptibility to drug-induced toxicity and may influence responsiveness to therapy and clinical response time in patients with IBD.

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