Current and Future Role of Serogenomics in Ulcerative Colitis

Mark H. Flasar, MD, MS, Raymond K. Cross, MD, MS, and David B. Doman, MD

Dr. Flasar is an Assistant Professor of Medicine in the Division of Gastroenterology and Hepatology at the University of Maryland School of Medicine and a staff physician in the Gastroenterology Section of the Veterans Affairs, Maryland Health Care System, both in Baltimore, Maryland.
Dr. Cross is an Associate Professor of Medicine in the Division of Gastroenterology and Hepatology at the University of Maryland School of Medicine and Chief of the Gastroenterology Section of the Veterans Affairs, Maryland Health Care System, both in Baltimore, Maryland.
Dr. Doman is a Clinical Professor of Medicine in the Division of Gastroenterology at George Washington University School of Medicine in Washington, DC.

Address correspondence to:
Dr. Mark H. Flasar
100 North Greene Street, Lower Level
Baltimore, MD 21201;
Tel: 410-706-3387;
Fax: 410-706-4330;
E-mail: mflasar@medicine.umaryland.edu

Keywords
Biologic markers, ulcerative colitis, serologic testing, genetic markers

Abstract: Ulcerative colitis (UC), a chronic inflammatory bowel disease, occurs in genetically susceptible individuals who mount inappropriate immune responses to endoluminal antigens. Serologic and genetic markers have shown great potential for clinical application in Crohn's disease (CD), particularly for prognostication. However, their use is not as well established in UC. The aim of this paper is to highlight the clinical relevance of these markers for diagnostics and prognostication in UC. This review identified studies that cited the use of serum and genetic biomarkers in UC when these biomarkers were used in diagnostic, prognostic, and therapeutic response prediction applications. Several serologic and genetic markers associated with UC were identified, and this review presents and summarizes these data, focusing on the biomarkers' established and emerging diagnostic and prognostic utility. Although more established in CD, the data provided by serologic and genetic testing in UC has the potential to enhance clinical decision making.

Ulcerative colitis (UC) is a chronic relapsing and remitting inflammatory disease of the large intestine that is thought to result from a dysregulated immune response to intestinal flora in genetically susceptible individuals.[1] Patients with UC present with a spectrum of phenotypic manifestations ranging from mild proctitis to severe pancolitis. Many patients with milder disease respond to traditional, conservative, step-up treatment approaches, while a smaller but significant subset of patients may be better served by accelerated step-up approaches or even earlier, more aggressive therapies, although the latter approach is controversial.[2] Clinical parameters such as symptom severity or endoscopic disease assessment are, at best, inexact prognostic guides for making informed, long-term therapeutic decisions. UC serogenomic profiles hold

the promise of patient stratification for optimal clinical management. In addition, as pharmacologic companies develop new biologic alternatives to anti–tumor necrosis factor therapy, newer-generation serogenomic profiles may serve to provide targeted pharmacologic therapeutic opportunities. This review will summarize the emerging diagnostic and prognostic utility of genetic markers for UC, as well as the utility of known serologic biomarkers.

Genetics of Ulcerative Colitis

Population-based and twin studies have long supported the notion of a genetic contribution to the development of UC.[3] Despite early findings confirming that the major histocompatibility complex region on chromosome 6 (encompassing the human leukocyte antigen [HLA] genes) is important in determining susceptibility for UC and associations with extensive disease, significant progress in clarifying and detailing this association had been relatively limited.[4,5] However, recent technologic advances have promoted rapid growth in the study of the genetics of UC.

The advent of genome-wide association studies (GWASs)—which analyze pooled DNA from numerous individuals to assess for single genetic mutations (called single-nucleotide polymorphisms [SNPs]) and associate them with various traits—has allowed researchers to identify 47 confirmed UC susceptibility loci (21 in UC alone and 26 in both UC and Crohn's disease [CD]).[4,6,7] Additional gene polymorphisms associated with UC are continuously being introduced, proving that the field continues to progress rapidly.[8] Studies have revealed that interleukin-23 receptor (IL-23R) and transcription factor genes that regulate cytokine expression represent the bulk of the associations common to both CD and UC; HLA, interleukin (IL)-10 signaling, and barrier function genes represent the bulk of the susceptibility loci unique to UC.[3,4] In addition to these loci having an association with the development or presence of UC, some studies have suggested that some of these loci are also linked to clinical factors.

Associations Between Genetics and Clinical Factors in Ulcerative Colitis

Several methods have been used to draw associations between genetic and clinical factors of UC. First, genome analysis of DNA extracted from tissue samples can provide clues as to whether fixed genetic information is associated with disease factors. Also, the presence of genome mutations in DNA from tissue samples—either mutations in a sequence or (once identified in a GWAS) a SNP—can be analyzed to assess for relevant associations. These methods essentially hint at what a cell, organism, or individual is capable of doing. In contrast, analysis of messenger RNA samples shows what that cell, organism, or individual is actually doing at the time of sampling, as this methodology (transcriptomics) analyzes products of transcription.

While not generalizable to all UC patients, a case-control analysis of 114 UC patients with colorectal cancer who were matched with 114 UC patients without cancer revealed several significant associations (both positive and negative) between specific HLA alleles and risk of colorectal cancer in UC patients.[9] The HLA DRB1*0103 allele has been associated with more extensive and refractory disease, shorter time to surgery, and extraintestinal manifestations (EIMs) in UC.[10,11] Interestingly, the HLA DRB1*0103 allele has also been associated with a colonic CD phenotype.[12-15] The barrier function gene *CDH1*, which encodes the transmembrane protein E-cadherin, was independently identified in separate GWASs for both UC and colorectal cancer, suggesting a possible link between UC and colonic dysplasia/neoplasia. In addition, promoter methylation of *CDH1* has been associated with dysplasia in UC patients, raising the possibility that hypermethylation might be used as a biomarker for the identification of UC patients who are at increased risk for dysplasia.[4] However, some studies have reported less promising associations. An analysis of inflammatory bowel disease (IBD)-associated IL-23R SNPs in UC patients revealed no association with disease extent, need for colectomy, or presence of EIMs.[16]

Despite these advances, the full clinical application of these genetic discoveries has yet to be realized. Identifying targets for the development of medical therapies is an obvious application of this new information, one that has already been met via development of therapies associated with IL-23R and IL-10.[4] Use of genetic information to develop diagnostic tests that could diagnose IBD and/or differentiate CD from UC is another attractive application. At the 2009 American College of Gastroenterology Annual Meeting, one study presented an analysis of the peripheral blood expression levels of 10 previously identified genes in a prospective cohort of 98 irritable bowel syndrome (IBS) patients and 189 IBD patients (91 UC and 98 CD). The study authors described an optimal scoring algorithm for classification of disease as IBS or IBD; this algorithm used 7 of the 10 tested genes and achieved 89% sensitivity and 74% specificity.[17]

The following year, this same group presented a UC/CD discrimination panel of 3 genes (*MMD*, *CD4*, and *DNAJA1*) that were identified by a proprietary analytic engine (Coperna, Exagen Diagnostics) using samples from 26 UC patients and 59 CD patients. The gene panel was then tested using peripheral blood from 192 IBD patients (97 CD and 95 UC). The authors

reported sensitivities for UC and CD of 87% and 92%, respectively; specificities were 92% and 87%, respectively. Positive and negative predictive values for both CD and UC ranged between 88% and 91%.[18] Both abstracts have yet to be published in full manuscript form but remain intriguing, despite their methodology limitations and the lack of validation in a low-prevalence cohort. The 2 genetic testing panels are currently commercially available and have been marketed as the first genomic tests for IBD, IBS, and UC/CD.[19]

A more recent study used a gene chip to determine the serologic gene expression profiles of 21 UC patients, 24 CD patients, and 10 control patients. The authors used logistic regression analyses to identify a set of 4 probes (IL receptor type II, cluster of differentiation 300A, karyopherin α 4, and embryonic lethal abnormal vision 1) that were reliable for differentiating UC from CD and control patients.[20] They concluded that these results support the practical use of serogenomic profiling as a diagnostic test in UC.

More stable and permanent than clinical, environmental, or serologic factors, genetic markers are an attractive potential alternative for predicting disease type, course, and outcome of therapy.[21] Data on the application of tissue-based genetic expression profiling have advanced steadily, while the less invasive serologic genetic testing methods have generally lagged behind.[22-24] Although serum-based genetic approaches have been used in concert with serologic markers in predicting the course of CD, predictive use of serum-based genetic markers for UC is limited.[21,25,26] A study in which previously identified UC SNPs were genotyped in 1,455 Dutch UC patients did not find an association between UC-specific loci and severity or extent of disease.[27] One GWAS comparing 324 medically refractory UC patients and 537 medically responsive UC patients found that a risk score based on the 46 SNPs associated with medically refractory UC accounted for nearly half of the risk of colectomy. The authors concluded that a SNP-based risk scoring system could prove useful in helping to predict which patients are likely to have the most severe disease course.[10] A smaller study examined 95 UC patients for the specific R72P polymorphism in the gene coding for the p53 tumor suppressor protein. The investigators found that, while this SNP was not associated with risk for UC, it was significantly associated with UC-associated colectomy and use of steroids.[28]

Prediction of response to therapy is another application of genetic testing, and much attention has been aimed at the multidrug resistance gene 1 (*MDR1*), which codes for an adenosine triphosphate binding protein that plays an important role in the pharmacokinetics of several medications. Specifically, *MDR1* codes for the drug efflux pump P-glycoprotein, a membrane transporter that lowers the intracellular concentration of glucocorticoids and has been associated with steroid-resistant UC.[29] Expression of *MDR1* has been shown to be low in the inflamed mucosa of UC patients, while specific SNPs of the *MDR1* gene have been associated with UC.[30,31] A recent study also showed that *MDR1* RNA expression from rectal biopsy specimens was significantly decreased in patients with active UC compared to UC patients in remission. Also, medical treatment response and long-term remission were both associated with high *MDR1* expression levels in this small cohort.[32] Interestingly, in a separate analysis of 154 steroid-refractory UC patients, specific *MDR1* SNPs were associated with higher resistance rates to rescue therapy with intravenous cyclosporine A.[33]

Kabakchiev and colleagues examined the peripheral blood RNA expression profiles of 20 steroid-responsive hospitalized pediatric UC patients and 20 steroid-resistant hospitalized pediatric UC patients on Day 3 following initiation of intravenous corticosteroids.[34] The researchers identified a total of 41 genes that were differentially expressed between responders and nonresponders, and they noted that matrix metallopeptidase 8 and carcinoembryonic antigen–related cell adhesion molecule 1 were both overexpressed in nonresponders. They also identified a cluster of 10 genes (from the 41 genes studied) that had a sensitivity of 80% and specificity of 80% for predicting response.[34]

Finally, in perhaps the most compelling example of how genetic markers can be combined with currently available clinical and serologic parameters in UC patients, investigators from Munich, Germany retrospectively assessed clinical activity, perinuclear antineutrophil cytoplasmic antibody (pANCA) status, and UC-specific IL-23R variants in 90 UC patients who were treated with infliximab (Remicade, Janssen Biotech) for 14 weeks. This multivariate analysis suggested that pretreatment pANCA seronegativity and the presence of IBD risk–increasing IL-23R variants were associated with a higher rate of response to infliximab.[35] Given that microbial seroreactivity has been associated with pattern recognition receptor genes, serologic testing is likely to be a temporary diagnostic and prognostic bridge to eventual genetic testing.[21,36,37] During this transition period, panels that combine traditional and currently available genetic serologic testing seem to be most promising.

As the genetics contributing to the pathogenesis of UC continue to be determined at a rapid rate, the promise of using genetic testing to diagnose UC and to predict clinical course and response to therapy in UC is becoming a reality. Eventually, through a combination of clinical factors, traditional serologic tests, and serologic identification of UC-specific genetic polymorphisms,

providers will likely be able to predict which patients are at high risk for colectomy and to prescribe highly effective, well-tolerated therapies.

Serologic Markers in Ulcerative Colitis

In general, serologic markers refer to distinct antibodies directed against different luminal antigens. The pattern and degree of positivity of these antibodies in individual patients are felt to represent a spectrum of immune phenotypes, which can theoretically be used to help differentiate patients based on disease type, phenotype, behavior, and prognosis. Thus, serologic markers are felt to represent an intermediary between genetic markers and the overt clinical symptoms that will eventually develop. However, concerns about the long-term stability of serologic profiles have been raised, in part from observations that seropositivity was lost in CD patients following initiation of a gluten-free diet.[38] Also, because of limitations in the CD and UC populations studied to date and the resultant limitations of the test characteristics of the antibody panels, caution is warranted when utilizing serologic testing as a primary diagnostic tool.[39]

*Perinuclear Antineutrophil Cytoplasmic Antibodies and Anti–*Saccharomyces cerevisiae *Mannan Antibodies*

The earliest and most extensively studied IBD serologic markers are anti–*Saccharomyces cerevisiae* mannan antibodies (ASCA) and pANCA. ASCA is an antibody against mannan residues on the cell wall of the yeast *S. cerevisiae*. ASCA is typically thought of as the CD antibody, and elevated ASCA titers have been reported to be highly specific (although poorly sensitive) for identifying CD patients.[40] Atypical pANCA is an autoantibody directed against granules on the rim of neutrophil nuclei as well as intranuclear foci. Typically thought of as the UC antibody, pANCA titers are more frequently elevated in UC patients and CD patients with a UC-like phenotype than in typical CD patients or healthy controls.[40-44] Studies have reported that pANCA-positive UC patients are more refractory to medical therapy and have a higher likelihood of requiring surgery.[45] More recently, investigators reported that UC patients with backwash ileitis were more likely to exhibit high titers of pANCA than UC patients without backwash ileitis.[46] The reported test characteristics of a positive pANCA result for the identification of UC vary, with sensitivities of 50–67%, specificities of 75–94%, and positive predictive values of 72–93%.[47-50] Both pANCA and ASCA tests are widely available; however, it has been suggested that, while ASCA results are fairly stable across different laboratories, pANCA test characteristics can vary widely.[50]

IBD type-unclassified (IBD-U), previously called indeterminate colitis, describes a group of patients with IBD colitis who cannot be classified as having UC or CD based on standard diagnostic testing. As this subgroup of patients represents up to 10% of patients with IBD and determination of a patient's true IBD subtype has therapeutic and prognostic implications, use of serologic testing to help clarify this gray area has been avidly pursued. Several studies suggest that the absence of ASCA in a pANCA-positive patient increases the specificity for UC while decreasing the sensitivity for UC.[47-50] A meta-analysis of 60 studies combining ASCA and pANCA testing in 4,019 UC patients, 3,841 CD patients, and 3,748 control patients reported similar results in terms of the ability of testing to differentiate subtypes of UC from CD, with a sensitivity of 51% and a specificity of 94%. Interestingly, the accuracy of a pANCA-positive/ASCA-negative result in identifying UC was more pronounced in a pediatric subgroup.[51]

More specifically addressing the question of how testing can clarify a diagnosis of IBD-U, Joossens and coauthors assessed ASCA and pANCA status in a cohort of 97 IBD-U patients who were followed prospectively.[52] Nearly half of the overall cohort was seronegative for both antibodies at baseline; most of these patients retained the diagnosis of IBD-U on follow-up examination. Thirty-two percent of patients were clinically diagnosed with UC or CD over a mean follow-up period of nearly 10 years. Of the 26 patients who were ASCA-positive/pANCA-negative, 8 were clinically diagnosed with CD during the follow-up period, and 2 were clinically diagnosed with UC. Of the 20 patients who were ASCA-negative/pANCA-positive, 7 were clinically diagnosed with UC during the follow-up period, and 4 were clinically diagnosed with CD. Interestingly, these CD patients had a UC-like phenotype. The overall results of this study did not support using ASCA and pANCA testing to differentiate CD or UC in IBD-U patients.

Prediction of disease course is another potential application of serologic testing. Use of serologic biomarkers to predict disease course, clinical relapse, and response to therapy has undergone far more development in CD than UC, although some data are available regarding serologic biomarkers in UC (Table 1).[53] In an analysis of predictors of early response to infliximab in UC patients, Ferrante and colleagues found that only 55% of ASCA-negative/pANCA-positive UC patients exhibited early clinical response, compared to 76% of patients without this antibody response pattern.[54] Similarly, a more recent pediatric study found reduced response rates (29%) to infliximab in pANCA-positive UC patients.[55] A retrospective analysis that pooled 56 left-sided UC patients from 4 separate clinical trials reported that treatment-resistant patients had a higher frequency of pANCA positivity than treatment-respon-

Table 1. Studies Showing an Association Between Perinuclear Antineutrophil Cytoplasmic Antibody (pANCA) Serologic Status and Response to Therapy in Ulcerative Colitis Patients

Serologic pattern	Year	Authors	Association
pANCA-positive	1996	Sandborn WJ, et al[45]	Increased rates of medically refractory disease; increased colectomy risk
pANCA-positive	2010	Dubinsky MC, et al[55]	Decreased infliximab response
pANCA-positive	2008	Hoie O, et al[56]	Increased rates of relapse; increased risk of relapse
pANCA-positive/ASCA-negative	2007	Ferrante M, et al[54]	Decreased early infliximab response
pANCA-negative/high IBD-risk IL-23R polymorphism	2010	Jurgens M, et al[35]	Increased infliximab response

ASCA=anti–*Saccharomyces cerevisiae* mannan antibodies; IBD=inflammatory bowel disease; IL-23R=interleukin-23 receptor.

sive patients (90% vs 60%).[45] Finally, while colectomy rates were not associated with pANCA status in a population-based, Norwegian, UC cohort of 432 patients, pANCA-positive patients had a 40% higher risk of relapse over 10 years than pANCA-negative patients.[56] These findings, along with others, suggest that pANCA-negative UC patients represent a subgroup that is more likely to respond to therapy, thus setting the stage for possible use of pANCA status to select between aggressive and traditional treatment algorithms.

Studies have also assessed the utility of serologic testing to predict the development of complications following colectomy with ileal pouch–anal anastomosis (IPAA) for presumed UC or IBD-U. Early studies, which suffered from significant design heterogeneity, inconsistently associated pANCA seropositivity with the development of pouchitis following IPAA.[57-61] In the first study to assess pANCA levels prior to colectomy, Fleshner and coauthors reported that pouchitis developed more frequently in patients who were pANCA-positive preoperatively.[62] Further, they noted an increased risk of chronic pouchitis in patients with high pANCA titers, and they found high pANCA titers to be the only independent risk factor for the development of chronic pouchitis after IPAA for UC.[62] Subsequently, serology was utilized to predict other complications following IPAA. In an attempt to identify predictors of the development of ileal CD following IPAA, serologic analysis was performed on samples from 238 consecutive patients who underwent colectomy and IPAA for a diagnosis of UC or IBD-U. Sixteen patients developed CD after a median of 14 months following IPAA, and ASCA immunoglobulin (Ig)A seropositivity was found to be an independent risk factor for the development of CD.[63] A separate retrospective analysis of 34 patients with UC or IBD-U who underwent IPAA and had previously undergone ASCA/pANCA serologic testing found that ASCA-positive/pANCA-negative patients were significantly more likely than ASCA-negative/pANCA-positive patients to develop CD or pouch-associated fistulae following IPAA. No association between serologic response and pouchitis was identified.[64]

Related Biomarkers

Anti-OmpC is directed against a 35-kDa *Escherichia coli* outer membrane porin and exhibits cross-reactivity with pANCA. Early research suggested a possible diagnostic role of anti-OmpC in UC patients, especially those with high pANCA titers.[65] However, subsequent use of anti-OmpC in addition to pANCA and ASCA in a population of IBD and control patients suggested that the utility of anti-OmpC may be limited to increasing the sensitivity of a panel of antibodies for the detection of CD.[66]

Anti-I2 is directed against a *Pseudomonas fluorescens*–associated DNA sequence detectable within the mucosa of IBD patients. This finding is more frequent in CD patients, as is the presence of a detectable serologic response.[67] A small pediatric study did not find I2 reactivity to be helpful in distinguishing between CD and UC, despite higher reported positivity in UC patients in this study than in adult studies.[68] Unfortunately, additional testing for OmpC and I2 has shown that these antibodies have minimal incremental benefit over ASCA/pANCA in differentiating IBD-U.[69] Finally, Hui and colleagues analyzed the ASCA, OmpC, and I2 serologic profiles of 28 IBD-U patients to assess the development of CD or chronic pouchitis following IPAA.[70] While they did not find the individual antibodies to be predictive, they did report a significantly greater frequency of chronic pouchitis in patients with any positive antibody compared to patients with no seroreactivity (63% vs 17%).[70]

Serologic response to flagellin (CBir1), which was identified in the C3H/HeJBir mouse model as a dominant antigen capable of inducing colitis in mice, has been found to be significantly higher in CD patients than UC patients.[71] While anti-CBir1 reactivity is more common in pANCA-positive CD patients than pANCA-positive UC patients, its utility seems greater for identifying CD patients who are at risk for complicated CD.[72] However, CBir1 positivity may increase the risk for the development of acute and chronic pouchitis following IPAA in UC patients, although this risk seems to be modulated by the corresponding pANCA titers.[73]

Antiglycans

As previously discussed, ASCA is an antiglycan antibody directed against mannan residues located on the cell surface of *S. cerevisiae*. Additional antibodies directed against sugars (glycans) on the cell surface of microbes as well as immune cells have been used to identify IBD patients; these antibodies include anti–laminaribioside carbohydrate IgG (ALCA), anti–chitobioside carbohydrate IgA (ACCA), and anti–mannobioside carbohydrate IgG (AMCA).[53] While these antibodies may be useful in identifying CD patients, early studies did not find a correlation between antiglycan antibodies and UC.[74] Likewise, an analysis of 40 IBD-U patients by Ferrante and coworkers revealed that an assay of g-ASCA (a second-generation ASCA), ALCA, and pANCA offered no clinically relevant improvement over traditional ASCA/pANCA testing in differentiating CD from UC.[75] However, a subsequent analysis of 818 IBD patients—which used 2 newer antiglycans (anti-L and anti-C) in addition to the standard panel (ACCA, ALCA, AMCA, and pANCA)—suggested additional diagnostic utility. While the majority (72%) of UC patients did not exhibit any of the antibodies on the panel, use of anti-C and anti-L increased the test's ability to discriminate between CD and UC, as well as between isolated colonic CD and UC, compared to testing with ASCA and pANCA alone.[76]

While traditional serologic testing is more readily available and broadly studied than serologic genetic testing, the practical application of traditional serologic testing in the diagnosis, differentiation, and prediction of UC is limited by proprietary laboratory methodology, test characteristics that depend strongly on disease prevalence, and inconsistent study results. While data suggest that pANCA-positive patients may represent a group that is more resistant to therapy and has an increased risk for chronic pouchitis following IPAA, traditional serologic markers need to be combined with additional serologic or serogenomic markers in order for the full potential of prediction and personalized medicine in UC to be realized.

Conclusion

Although dwarfed by the advances seen in CD, the diagnostic and prognostic potential of genetic and serum biomarkers in UC is increasing. As the growth of UC genetic testing moves from infancy to adolescence, traditional serum biomarkers are primarily being used to help confirm a UC diagnosis, to distinguish between UC and CD, and to predict clinical course and response to therapy. However, there is clearly room for improvement with the addition of genetic testing or identification of other serologic markers. Multiple UC-associated genes have already been identified, some of which may provide targets for future therapeutics—a strategy that has already been utilized in the development of IL-23R antagonists. In addition, genetic markers may help to identify patients who are at risk for more severe or refractory disease, dysplasia, or colorectal cancer. Such patient identification may allow selection of patients for earlier, more aggressive therapy. Serum genetic profiles may also be used to predict response and tolerability to therapy, thus allowing healthcare providers to personalize medication choices to optimize outcomes. Finally, as pharmacologic companies develop new therapies, future UC serogenomic profiles offer the promise of targeted pharmacogenomic opportunities.

Dr. Flasar has nothing to disclose. Dr. Cross has received grant support from Prometheus Laboratories. Dr. Doman is on the speakers' bureau for Prometheus Laboratories.

References

1. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature*. 2007;448:427-434.
2. Panccione R, Ghosh S, Middleton S, et al. Infliximab, azathioprine, or infliximab + azathioprine for treatment of moderate to severe ulcerative colitis: the UC SUCCESS trial. Presented at Digestive Disease Week 2011; May 7–10, 2011; Chicago, IL. Abstract 835.
3. Cho JH, Brant SR. Recent insights into the genetics of inflammatory bowel disease. *Gastroenterology*. 2011;140:1704-1712.
4. Thompson AI, Lees CW. Genetics of ulcerative colitis. *Inflamm Bowel Dis*. 2011;17:831-848.
5. Satsangi J, Welsh KI, Bunce M, et al. Contribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. *Lancet*. 1996;347:1212-1217.
6. McGovern DP, Gardet A, Torkvist L, et al. Genome-wide association identifies multiple ulcerative colitis susceptibility loci. *Nat Genet*. 2010;42:332-337.
7. Anderson CA, Boucher G, Lees CW, et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. *Nat Genet*. 2011;43:246-252.
8. Muise AM, Walters T, Xu W, et al. Single nucleotide polymorphisms that increase expression of the guanosine triphosphatase RAC1 are associated with ulcerative colitis. *Gastroenterology*. 2011;141:633-641.
9. Garrity-Park MM, Loftus EV Jr, Sandborn WJ, Bryant SC, Smyrk TC. MHC Class II alleles in ulcerative colitis-associated colorectal cancer. *Gut*. 2009;58:1226-1233.
10. Haritunians T, Taylor KD, Targan SR, et al. Genetic predictors of medically refractory ulcerative colitis. *Inflamm Bowel Dis*. 2010;16:1830-1840.
11. Ahmad T, Armuzzi A, Neville M, et al. The contribution of human leucocyte antigen complex genes to disease phenotype in ulcerative colitis. *Tissue Antigens*. 2003;62:527-535.

12. Roussomoustakaki M, Satsangi J, Welsh K, et al. Genetic markers may predict disease behavior in patients with ulcerative colitis. *Gastroenterology*. 1997;112:1845-1853.
13. Cassinotti A, Birindelli S, Clerici M, et al. HLA and autoimmune digestive disease: a clinically oriented review for gastroenterologists. *Am J Gastroenterol*. 2009;104:195-217; quiz 194, 218.
14. Silverberg MS, Mirea L, Bull SB, 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.
15. Fisher SA, Tremelling M, Anderson CA, et al. Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. *Nat Genet*. 2008;40:710-712.
16. Cummings JR, Ahmad T, Geremia A, et al. Contribution of the novel inflammatory bowel disease gene IL23R to disease susceptibility and phenotype. *Inflamm Bowel Dis*. 2007;13:1063-1068.
17. Alsobrook J 2nd, Davis L, Williams T, Leighton J, Harris C. Accurate differentiation between irritable bowel syndrome and inflammatory bowel disease using novel biomarkers from peripheral blood specimens. *Am J Gastroenterol*. 2009;104:S494.
18. Harris C, Ma T, Leighton J, et al. Accurate differentiation between Crohn's disease and ulcerative colitis in inflammatory bowel disease using novel biomarkers from peripheral blood specimens. *Am J Gastroenterol*. 2010;105:S461.
19. Exagen Diagnostics. http://exagen.com/. Accessed July 2011.
20. Burakoff R, Chao S, Perencevich M, et al. Blood-based biomarkers can differentiate ulcerative colitis from Crohn's disease and noninflammatory diarrhea. *Inflamm Bowel Dis*. 2011;17:1719-1725.
21. Vermeire S, Van Assche G, Rutgeerts P. Role of genetics in prediction of disease course and response to therapy. *World J Gastroenterol*. 2010;16:2609-2615.
22. Arijs I, Li K, Toedter G, et al. Mucosal gene signatures to predict response to infliximab in patients with ulcerative colitis. *Gut*. 2009;58:1612-1619.
23. Watson AJ, Tremelling M. Mucosal gene expression signatures that predict response of ulcerative colitis to infliximab. *Gastroenterology*. 2011;140:729-731.
24. Toedter G, Li K, Marano C, et al. Gene expression profiling and response signatures associated with differential responses to infliximab treatment in ulcerative colitis. *Am J Gastroenterol*. 2011;106:1272-1280.
25. Lichtenstein GR, Targan SR, Dubinsky MC, et al. Combination of genetic and quantitative serological immune markers are associated with complicated Crohn's disease behavior. *Inflamm Bowel Dis*. 2011 Mar 9. Epub ahead of print.
26. Tamboli CP, Doman DB, Patel A. Current and future role of biomarkers in Crohn's disease risk assessment and treatment. *Clin Exp Gastroenterol*. 2011;4:127-140.
27. Festen EA, Stokkers PC, van Diemen CC, et al. Genetic analysis in a Dutch study sample identifies more ulcerative colitis susceptibility loci and shows their additive role in disease risk. *Am J Gastroenterol*. 2010;105:395-402.
28. Eren F, Akkiprik M, Atug O, et al. R72P polymorphism of TP53 in ulcerative colitis patients is associated with the incidence of colectomy, use of steroids and the presence of a positive family history. *Pathol Oncol Res*. 2010;16:563-568.
29. De Iudicibus S, Franca R, Martelossi S, Ventura A, Decorti G. Molecular mechanism of glucocorticoid resistance in inflammatory bowel disease. *World J Gastroenterol*. 2011;17:1095-1108.
30. Potocnik U, Ferkolj I, Glavac D, Dean M. Polymorphisms in multidrug resistance 1 (MDR1) gene are associated with refractory Crohn disease and ulcerative colitis. *Genes Immun*. 2004;5:530-539.
31. Ho GT, Nimmo ER, Tenesa A, et al. Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. *Gastroenterology*. 2005;128:288-296.
32. Yamamoto-Furusho JK, Villeda-Ramirez MA, Fonseca-Camarillo G, et al. High gene expression of MDR1 (ABCB1) is associated with medical treatment response and long-term remission in patients with ulcerative colitis. *Inflamm Bowel Dis*. 2010;16:541-542.
33. Daniel F, Loriot MA, Seksik P, et al. Multidrug resistance gene-1 polymorphisms and resistance to cyclosporine A in patients with steroid resistant ulcerative colitis. *Inflamm Bowel Dis*. 2007;13:19-23.
34. Kabakchiev B, Turner D, Hyams J, et al. Gene expression changes associated with resistance to intravenous corticosteroid therapy in children with severe ulcerative colitis. *PLoS One*. 2010;5:e13085.
35. Jurgens M, Laubender RP, Hartl F, et al. Disease activity, ANCA, and IL23R genotype status determine early response to infliximab in patients with ulcerative colitis. *Am J Gastroenterol*. 2010;105:1811-1819.
36. Devlin SM, Yang H, Ippoliti A, et al. NOD2 variants and antibody response to microbial antigens in Crohn's disease patients and their unaffected relatives. *Gastroenterology*. 2007;132:576-586.
37. Henckaerts L, Pierik M, Joossens M, Ferrante M, Rutgeerts P, Vermeire S. Mutations in pattern recognition receptor genes modulate seroreactivity to microbial antigens in patients with inflammatory bowel disease. *Gut*. 2007;56:1536-1542.
38. Papp M, Foldi I, Altorjay I, et al. Anti-microbial antibodies in celiac disease: trick or treat? *World J Gastroenterol*. 2009;15:3891-3900.
39. Austin GL, Shaheen NJ, Sandler RS. Positive and negative predictive values: use of inflammatory bowel disease serologic markers. *Am J Gastroenterol*. 2006;101:413-416.
40. Ruemmele FM, Targan SR, Levy G, Dubinsky M, Braun J, Seidman EG. Diagnostic accuracy of serological assays in pediatric inflammatory bowel disease. *Gastroenterology*. 1998;115:822-829.
41. Saxon A, Shanahan F, Landers C, Ganz T, Targan S. A distinct subset of antineutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. *J Allergy Clin Immunol*. 1990;86:202-210.
42. Cambridge G, Rampton DS, Stevens TR, McCarthy DA, Kamm M, Leaker B. Anti-neutrophil antibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut*. 1992;33:668-674.
43. Duerr RH, Targan SR, Landers CJ, Sutherland LR, Shanahan F. Anti-neutrophil cytoplasmic antibodies in ulcerative colitis. Comparison with other colitides/diarrheal illnesses. *Gastroenterology*. 1991;100:1590-1596.
44. Oberstadt K, Schaedel W, Weber M, Classen M, Deusch K. P-ANCA as a differential diagnostic marker in inflammatory bowel disease. *Adv Exp Med Biol*. 1995;371B:1313-1316.
45. Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Association of antineutrophil cytoplasmic antibodies with resistance to treatment of left-sided ulcerative colitis: results of a pilot study. *Mayo Clin Proc*. 1996;71:431-436.
46. White E, Melmed GY, Vasiliauskas EA, et al. A prospective analysis of clinical variables, serologic factors, and outcome of ileal pouch-anal anastomosis in patients with backwash ileitis. *Dis Colon Rectum*. 2010;53:987-994.
47. Quinton JF, Sendid B, Reumaux D, et al. Anti-*Saccharomyces cerevisiae* mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut*. 1998;42:788-791.
48. Peeters M, Joossens S, Vermeire S, Vlietinck R, Bossuyt X, Rutgeerts P. Diagnostic value of anti-*Saccharomyces cerevisiae* and antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. *Am J Gastroenterol*. 2001;96:730-734.
49. Koutroubakis IE, Petinaki E, Mouzas IA, et al. Anti-*Saccharomyces cerevisiae* mannan antibodies and antineutrophil cytoplasmic autoantibodies in Greek patients with inflammatory bowel disease. *Am J Gastroenterol*. 2001;96:449-454.
50. Sandborn WJ, Loftus EV Jr, Colombel JF, et al. Evaluation of serologic disease markers in a population-based cohort of patients with ulcerative colitis and Crohn's disease. *Inflamm Bowel Dis*. 2001;7:192-201.
51. Reese GE, Constantinides VA, Simillis C, et al. Diagnostic precision of anti-*Saccharomyces cerevisiae* antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol*. 2006;101:2410-2422.
52. Joossens S, Reinisch W, Vermeire S, et al. The value of serologic markers in indeterminate colitis: a prospective follow-up study. *Gastroenterology*. 2002;122:1242-1247.
53. Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. *Gastroenterology*. 2011;140:1817-1826.
54. Ferrante M, Vermeire S, Katsanos KH, et al. Predictors of early response to infliximab in patients with ulcerative colitis. *Inflamm Bowel Dis*. 2007;13:123-128.
55. Dubinsky MC, Mei L, Friedman M, et al. Genome wide association (GWA) predictors of anti-TNFalpha therapeutic responsiveness in pediatric inflammatory bowel disease. *Inflamm Bowel Dis*. 2010;16:1357-1366.
56. Hoie O, Aamodt G, Vermeire S, et al. Serological markers are associated with disease course in ulcerative colitis. A study in an unselected population-based cohort followed for 10 years. *J Crohns Colitis*. 2008;2:114-122.
57. Sandborn WJ, Landers CJ, Tremaine WJ, Targan SR. Antineutrophil cytoplasmic antibody correlates with chronic pouchitis after ileal pouch-anal anastomosis. *Am J Gastroenterol*. 1995;90:740-747.
58. Kaditis AG, Perrault J, Sandborn WJ, Landers CJ, Zinsmeister AR, Targan SR. Antineutrophil cytoplasmic antibody subtypes in children and adolescents after ileal pouch-anal anastomosis for ulcerative colitis. *J Pediatr Gastroenterol Nutr*. 1998;26:386-392.
59. Esteve M, Mallolas J, Klaassen J, et al. Antineutrophil cytoplasmic antibodies in sera from colectomised ulcerative colitis patients and its relation to the presence of pouchitis. *Gut*. 1996;38:894-898.
60. Yang P, Oresland T, Jarnerot G, Hulten L, Danielsson D. Perinuclear antineutrophil cytoplasmic antibody in pouchitis after proctocolectomy with ileal pouch-anal anastomosis for ulcerative colitis. *Scand J Gastroenterol*. 1996;31:594-598.
61. Yasuda N, Thomas P, Ellis H, Herbst F, Nicholls J, Ciclitira P. Perinuclear antineutrophil cytoplasmic antibodies in ulcerative colitis after restorative proctocolectomy do not correlate with the presence of pouchitis. *Scand J Gastroenterol*. 1998;33:509-513.
62. Fleshner PR, Vasiliauskas EA, Kam LY, et al. High level perinuclear antineutrophil cytoplasmic antibody (pANCA) in ulcerative colitis patients before colectomy predicts the development of chronic pouchitis after ileal pouch-anal anastomosis. *Gut*. 2001;49:671-677.

63. Melmed GY, Fleshner PR, Bardakcioglu O, et al. Family history and serology predict Crohn's disease after ileal pouch-anal anastomosis for ulcerative colitis. *Dis Colon Rectum*. 2008;51:100-108.
64. Dendrinos KG, Becker JM, Stucchi AF, Saubermann LJ, LaMorte W, Farraye FA. Anti-*Saccharomyces cerevisiae* antibodies are associated with the development of postoperative fistulas following ileal pouch-anal anastomosis. *J Gastrointest Surg*. 2006;10:1060-1064.
65. Cohavy O, Bruckner D, Gordon LK, et al. Colonic bacteria express an ulcerative colitis pANCA-related protein epitope. *Infect Immun*. 2000;68:1542-1548.
66. Zholudev A, Zurakowski D, Young W, Leichtner A, Bousvaros A. Serologic testing with ANCA, ASCA, and anti-OmpC in children and young adults with Crohn's disease and ulcerative colitis: diagnostic value and correlation with disease phenotype. *Am J Gastroenterol*. 2004;99:2235-2241.
67. Sutton CL, Kim J, Yamane A, et al. Identification of a novel bacterial sequence associated with Crohn's disease. *Gastroenterology*. 2000;119:23-31.
68. Iltanen S, Tervo L, Halttunen T, et al. Elevated serum anti-I2 and anti-OmpW antibody levels in children with IBD. *Inflamm Bowel Dis*. 2006;12:389-394.
69. Joossens S, Colombel JF, Landers C, et al. Anti-outer membrane of porin C and anti-I2 antibodies in indeterminate colitis. *Gut*. 2006;55:1667-1669.
70. Hui T, Landers C, Vasiliauskas E, et al. Serologic responses in indeterminate colitis patients before ileal pouch-anal anastomosis may determine those at risk for continuous pouch inflammation. *Dis Colon Rectum*. 2005;48:1254-1262.
71. Lodes MJ, Cong Y, Elson CO, et al. Bacterial flagellin is a dominant antigen in Crohn disease. *J Clin Invest*. 2004;113:1296-1306.
72. Targan SR, Landers CJ, Yang H, et al. Antibodies to CBir1 flagellin define a unique response that is associated independently with complicated Crohn's disease. *Gastroenterology*. 2005;128:2020-2028.
73. Fleshner P, Ippoliti A, Dubinsky M, et al. Both preoperative perinuclear anti-neutrophil cytoplasmic antibody and anti-CBir1 expression in ulcerative colitis patients influence pouchitis development after ileal pouch-anal anastomosis. *Clin Gastroenterol Hepatol*. 2008;6:561-568.
74. Dotan I, Fishman S, Dgani Y, et al. Antibodies against laminaribioside and chitobioside are novel serologic markers in Crohn's disease. *Gastroenterology*. 2006;131:366-378.
75. Ferrante M, Henckaerts L, Joossens M, et al. New serological markers in inflammatory bowel disease are associated with complicated disease behaviour. *Gut*. 2007;56:1394-1403.
76. Seow CH, Stempak JM, Xu W, et al. Novel anti-glycan antibodies related to inflammatory bowel disease diagnosis and phenotype. *Am J Gastroenterol*. 2009;104:1426-1434.