Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2008 January 21; 14(3): 338-347
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Daniel C Baumgart, MD, PhD, *Series Editor*

Inflammatory bowel disease: Genetic and epidemiologic considerations

Judy H Cho

Judy H Cho, Director, Inflammatory Bowel Disease Center, Yale University, New Haven, CT 06519, United States
Correspondence to: Judy H Cho, MD, Associate Professor of Medicine and Genetics, Director, Inflammatory Bowel Disease Center, Yale University, 300 Cedar St., S155A, New Haven, CT 06519, United States. judy.cho@yale.edu
Telephone: +1-203-7852052 Fax: +1-203-7857273
Received: May 16, 2007 Revised: July 4, 2007

Abstract

Genome-wide association studies have firmly established that many genomic loci contribute to inflammatory bowel disease, especially in Crohn's disease. These studies have newly-established the importance of the interleukin 23 and autophagy pathways in disease pathogenesis. Future challenges include: (1) the establishment of precisely causal alleles, (2) definition of altered functional outcomes of associated and causal alleles and (3) integration of genetic findings with environmental factors.

Key words: Crohn's disease; Ulcerative colitis; Interleukin 23; Autophagy; Complex genetics

Cho JH. Inflammatory bowel disease: Genetic and epidemiologic considerations. *World J Gastroenterol* 2008; 14(3): 338-347 Available from: URL: http://www.wjgnet.com/1007-9327/14/338.asp DOI: http://dx.doi.org/10.3748/wjg.14.338

INTRODUCTION

The fields of genetics and epidemiology share a common goal of identifying specific factors, present in some individuals, and absent in others, that contribute to disease causation. As with many diseases, in inflammatory bowel disease (IBD), carefully performed epidemiologic studies provide an ongoing basis for the rationale and design of genetic studies. Observations on the nature and magnitude of familial clustering of IBD cases provided the framework for the present genetic observations that Crohn's disease (CD) and ulcerative colitis (UC) share some susceptibility alleles (e.g. *IL23R*) whereas others are unique to one disease subtype or the other. This review summarizes present understanding of the genetics and epidemiology of IBD, and describes the encouraging early results from genome-wide association studies which demonstrate significant consistency between studies.

EPIDEMIOLOGIC OBSERVATIONS FORM THE BASIS FOR GENETIC STUDIES IN IBD

Familial risk in IBD

The observation that cases of IBD cluster within families suggested that both shared genetic and environmental factors could contribute to disease pathophysiology. That monozygotic (MZ) twin concordance is significantly higher than dizygotic (DZ) twin concordance for both CD (20%-50% *vs* 0%-7%, respectively) and UC (14%-19% *vs* 0%-5%, respectively) indicates that genetic factors definitively contribute to both disorders[1-4]. The observation that, even among MZ twins, disease concordance is significantly less than 100% demonstrates the significant role that environmental and developmental factors play in disease pathogenesis. Like most genetic disorders, inheritance in IBD does not follow a simple Mendelian pattern. This would indicate that no single gene mutation is sufficient and/or necessary to contribute to disease, indicating that IBD represents complex, multigenic genetic disorders. In complex genetic disorders, any single gene mutation/polymorphism will not confer a simple determinative effect of disease development (as is the case for Mendelian, single-gene disorders), but rather merely confers a statistically increased, but non-determinative susceptibility to developing disease. Approximately 5% to 20% of patients with IBD have a family history of IBD[5,6]. A family history of IBD is generally more prevalent among CD than UC patients[6]. The relative risk to siblings, λs, (sibling risk compared to general population risk) ranges from 30-40 for CD and 10-20 for UC[7,8]. Furthermore, the relative risk for UC to siblings of a CD proband was observed to be 3.9 and the cross-disease relative risk to siblings of a UC proband was observed to be 1.8, suggesting that CD and UC are genetically related[9].

Comparative prevalence of IBD

The prevalence of CD and UC is highest in North America, northern Europe, and the United Kingdom, with averages ranging from 100 to 200 cases per 100 000[10-13]. IBD is more common in European Americans compared with African Americans, and the lowest rates of IBD have been reported in Hispanics and Asians[7,14-17]. The

prevalence of CD based on inpatient and outpatient visits in a Southern California Kaiser HMO study was 43.6 per 100 000 for European Americans, 29.8 per 100 000 for African-Americans, 5.6 per 100 000 for Asians and 4.1 per 100 000 for Hispanics[18]. However, two different Kaiser HMO studies found similar rates of hospitalizations among African-American and European ancestry individuals[18,19]. In addition, a more recent study demonstrated a similar incidence of IBD in African-Americans and European Americans[20]. Within European ancestry populations, the rates of IBD are higher in persons of Jewish ancestry than other ethnic groups[14,21], and higher in Ashkenazi (Central and Eastern European) Jews than in Sephardic (Middle Eastern and Spanish ancestry) Jews[14,21].

The hygiene hypothesis

Generally, IBD incidence is lower in developing countries and higher in industrialized countries, such as in North America and Europe; as a country undergoes the transition to industrialization, the prevalence of UC rises first, followed by a rise in the prevalence of CD[10]. The hygiene hypothesis proposes that lack of exposure to select microbial agents in childhood causes IBD[22]. In support of this, in Manitoba, patients with CD are less likely to have lived on a farm and less likely to have drunk unpasteurized milk. Furthermore, CD patients were more likely to have used tap water rather than well water, and more likely to have had a pet[23]. Against this hypothesis, a separate study of pediatric IBD observed that owning a pet, day-care attendance, and "physician diagnosed infection" between 5 and 10 years of age was associated with increased CD risk, and regular use of a personal towel and lesser crowding in the home with decreased risk[24].

The measurable changes in disease incidence in various geographic regions highlights the contributory role of environmental factors in IBD pathogenesis. A leading culprit for such environmental contributions is a changing intestinal intraluminal microbial milieu. At birth, the intestinal lumen is largely sterile[25], with rapid bacterial colonization ensuing in the neonatal period. There is some evidence to suggest that fecal colonization patterns may reflect environmental cues in the perinatal period and thus may be manipulable[26,27] as a potential means of preventing disease in high risk individuals.

Tobacco and IBD

Within Western countries, smoking is the most well established environmental risk factor, increasing risk for CD by approximately two-fold[28] and non-smoking decreasing risk for UC two- to five-fold[14]. Being an ex-smoker increases the risk of developing UC two-fold[29]. Ex-smokers make up an increasing percentage of older patients diagnosed with UC, accounting for more than 35% of the attributable risk of late onset (> 45 years) UC and a large component of the second peak in diagnosis[30]. Interestingly, Bridger *et al* observed that in IBD pedigrees with UC/CD sibling pairs, smokers tend to develop CD and non-smokers UC (O.R. 10.5)[31]. A significant increase in smoking in younger patients with familial CD has been reported[30]. Smoking is also associated with early recurrence of CD following surgery[32]. Future genetic studies in IBD will explore whether specific genetic associations will be stratifiable based on tobacco status at the time of diagnosis. However, tobacco may exert many of its phenotypic effects through epigenetic (reversible, changes in gene regulation that occur without a change in DNA sequence or genotype) mechanisms, such as DNA methylation effects on transcriptional activation[33].

HUMAN GENETIC VARIATION AND THE HAPMAP PROJECT

The most abundant of the human genetic variants are single nucleotide polymorphisms (SNPs). A SNP is a DNA sequence variation occurring when a single nucleotide, A, T, C, or G, in the genome differs between individuals, or between homologous chromosomes within an individual. Three salient features of SNPs include, (1) their allele frequencies within a population of interest, (2) their correlation, or linkage disequilibrium, with SNPs in the immediate genomic vicinity, and (3) whether or not the SNP of interest results in a measurable phenotypic change in protein function and/or expression, that is, represents a functional polymorphism.

As a very general rule, SNPs with a minor allele frequency approaching 50%, are genetically more ancient, having had a longer time to increase in frequency, and are more likely to be observed in all racial groups. In contrast, uncommon SNPs having a minor allele frequency of less than 5% (that is 5 of 100 chromosomes tested from 50 individuals carry the minor allele) are more likely to be evolutionarily more recent, and are often observed uniquely in one racial group or another. A number of factors besides the evolutionary age of a SNP can affect its frequency within a population, notably whether or not the SNP confers a selective advantage or disadvantage with respect to reproductive fitness within a population. It may be speculated that functional SNPs that contribute to the development of chronic inflammatory disorders such as IBD may confer a selective advantage with respect to combating historically significant infectious pathogens.

It is estimated that there are 5 million SNPs common SNPs having a minor allele frequency greater than 10%[34]. In order to comprehensively sample all common variation throughout the genome, however, it is not required that millions of SNPs be directly tested, due to the high degree of correlation, or linkage disequilibrium that exists between SNPs throughout the human genome. The Human HapMap Project empirically defined the linkage disequilibrium patterns in European, African and Asian cohorts and provided the basis for the genome-wide association studies that increasingly being reported for various complex genetic disorders[35]. It is estimated that genotyping several hundred thousand SNPs in European ancestry cohorts will sample nearly 80% of the common human variation with an r^2 (measure of linkage disequilibrium) of greater than 0.8[36].

The costs of genotyping several hundred thousand SNPs continue to decrease, and therefore genotyping sufficiently large cohorts to identify IBD genes through genome-wide association studies is now feasible. Once

associations with SNPs are identified, the challenge remains to identify the functional polymorphisms that account for the statistical association. The precedent from Mendelian disorders would suggest that those amino acid variants which directly affect protein structure are likely to disproportionately contribute to disease susceptibility. Within amino acid polymorphisms, those variants in highly conserved (between species) regions or contained within key functional domains are more likely to have significant functional effects. Ultimately, however, proof of disease contribution requires demonstration of altered functional effects associated with the mutation of interest.

IBD GENETIC ASSOCIATIONS PRIOR TO THE ADVENT OF GENOME-WIDE ASSOCIATION STUDIES

Because the statistical effects observed in multigenic disorders are relatively modest, the hallmark for accepting genetic associations is replication in independent cohorts. However, interpretation of disease association studies has been complicated by the reporting of often conflicting studies. In such instances, meta-analyses of all reported studies can provide some insight, with the caveat that publication bias (e.g. negative studies may be more difficult to publish than positive ones) may occur.

Nod2 (CARD15) associations to ileal CD

The most well-replicated IBD genetic associations is the Nod2 gene association with ileal CD[37,38]. Nod2 was the first definitive risk factor for CD and one of the first genes identified for a common complex genetic disorder. It functions as an intracellular sensor for bacterial peptidoglycan[39], and can be activated by a minimal bioactive component, muramyl dipeptide (MDP)[40,41]. MDP is present in both gram positive and negative bacteria and activates NF-κB and MAP kinase pathways[42]. Three uncommon (minor allele frequency less than 5% in healthy controls) polymorphisms, Arg702Trp, Gly908Arg, and Leu1007fsinsC are each highly associated with CD. These findings have been extensively replicated by a number of subsequent studies. A meta-analysis of 39 studies showed an odds ratio for simple heterozygotes of 2.4 (confidence interval, C.I. 2.0-2.9), and for homozygous/compound heterozygous carriers of 17.1 (C.I. 10.7-27.2)[43]. Nod2 carriage has been specifically associated with ileal involvement, stricturing complications and a modestly earlier age of onset.

Each of the three CD polymorphisms are located within or near the leucine rich repeat, sensing domain of Nod2, and each results in a decreased capacity to activate NF-κB in response peptidoglycan or MDP stimulation[39,44-48]. The frameshift mutation, Leu1007fsinsC demonstrates a largely complete deficiency in the capacity to signal in response to MDP stimulation[45]. Therefore, these functional polymorphisms represent direct risk alleles for CD. Importantly, the Nod2 discovery provides specific support for the long-held hypothesis that CD results from a genetically dysregulated host immune response to luminal bacteria. These Nod2 mutations have not been observed in Japanese, Chinese and Koreans with IBD[49-51], and they are rare in African-American IBD[52].

Despite the high odds ratios associated with the Nod2 mutations, it is estimated that the disease penetrance, even for homozygous or compound heterozygous carriers is limited, suggesting that these Nod2 variants alone are insufficient to produce disease. As each of the three major mutations demonstrates decreased function in primary human cells[39,44-48], Nod2-deficient murine models provide an important model for human disease. The absence of intestinal inflammation in Nod2-deficient mice further highlights the insufficiency of this pathway alone to induce CD[42,53]. Since the discovery of the CD-association with Nod2, a number of studies have added to understanding of the Nod2 functional pathway.

Given the dysregulation in intestinal immune homeostasis characteristic of CD, it is logical to hypothesize that Nod2 is important for either impaired tolerance mechanisms critical in limiting excessive activation of the intestinal immune system and/or altered initial defenses against intestinal bacteria. There are a broad range of tolerance mechanisms operating in the intestine to regulate excessive immune responses in the context of its unique exposure to a continuous bacterial load. Regarding initial defenses, a defect in bacterial recognition and responses at the intestinal epithelial surface might result in alterations in the population of commensal organisms, and/or increased invasion, which in turn, could contribute to an increased propensity toward intestinal inflammation. Nod2 is highly expressed in Paneth cells of the small intestine[54,55] and Nod2 mutations are associated with ileal location of disease[56]. Studies in humans and mice have shown that normal Nod2 function is required for optimal defensin expression and therefore, impaired defensin expression and regulation may contribute to the increased CD susceptibility observed in Nod2 risk allele carriers[57-59].

IBD5 association on chromosome 5q31

Within the IBD5 linkage region, association of CD with a 250 kb region on chromosome 5q31 was reported[60]. This association has been subsequently replicated in a number of studies[61-64], for both CD and UC[62,65]. Candidate polymorphisms found on this haplotype within the organic cation transporter OCTN1 (*SLC22A4*) and OCTN2 (*SLC22A5*) genes have been reported[66,67]. However, statistically equivalent evidence for association has been observed throughout the risk haplotype[68] encompassing several genes, highlighting the need for additional genetic and functional investigation in this region. Phenotypic correlates have been reported for perianal disease[61,69], colonic location[70], disease complications and progression[68,71], extensive disease in UC[72], as well as for decreased height and weight at diagnosis in pediatric cohorts[73]. In contrast, some studies have not observed clear genotype-phenotype correlations[74,75].

HLA associations

Replicated HLA class Ⅱ associations in IBD include HLA-DRB1*1502 (serological marker HLA-DR2)[76] association with UC, and HLA-DRB1*0103 association with UC and colonic CD[77,78]. HLA-DRB1*0103 is noteworthy in that

it is a risk factor for both UC and colonic CD, suggesting it may play a role in chronic inflammation of the colon independent of major IBD phenotype (i.e., CD or UC)[78]. As with IBD5, the DRB1*0103 and DRB1*1502 class Ⅱ variants are in strong linkage disequilibrium with SNPs on multiple immunologically active candidate genes. Present approaches have not been able to discern whether these other genes or the class Ⅱ genes are the true risk genes in the HLA locus.

Reported, but unconfirmed IBD associations

Associations which have been reported within linkage regions to IBD but have not been consistently replicated include an indel polymorphism in the promoter region of the *NF-κB1* gene on chromosome 4q[79-82], multiple polymorphisms in the *MDR1/ABCB1* gene on chromosome 7q[83-92], and polymorphisms in the *DLG5* gene on chromosome 10q[70,93-102]. (See section C.4.1.) Haplotypes in the terminal exons of the chromosome 7p Nod1 (*CARD4*) gene and a Nod1 (*CARD4*) indel polymorphism were associated in two different study populations[47], although other studies have not replicated evidence for association[103-105]. Finally, studies of candidate genes that are not located within IBD linkage regions have revealed weak associations between UC and a variable number of tandem repeats polymorphism in the *IL1RN* gene[106] and between IBD and the TLR4 gene[107-113]. In a large case-control study, association was observed in UC and CD for Ala1011Ser within the Myo IXb (*MYO9B*) gene[114] that had been previously been implicated in celiac disease[115]. If replicated, this would indicate shared susceptibility pathways between multiple types of intestinal inflammation.

GENOME-WIDE ASSOCIATION STUDIES IN IBD

Emerging technologies now provide for the direct testing of a large number of SNPs throughout the genome in genome-wide association studies. The multiple genome-wide association studies reported below vary with respect to the genotyping platform utilized and the population cohort tested. Despite these differences, however, the first glimpses of comparative results between the genome-wide association studies are demonstrating encouraging consistency of findings between studies. Taken together, the early results from these studies would suggest that significant advances in defining well-replicated gene associations in IBD, especially in European ancestry cohorts, will shortly ensue.

TNFSF15 association to CD

The first genome-wide association study in IBD involved testing nearly 80 000 SNPs in a Japanese CD cohort[116]. The most significant findings implicated SNPs and haplotypes within the TNFSF15 (TNF superfamily) gene. These results were subsequently confirmed in European IBD cohorts[117]. TNFSF15 is a Th-1 polarizing cytokine which has been reported to be increased in IBD mucosa[118]. In a separate Belgian CD cohort, however, no significant evidence for association was observed[119]. These differences could reflect differences in markers genotyped and/or different contributions of susceptibility genes between Asian and European IBD cohorts. Future studies in a variety of population cohorts will provide important comparative insight.

IL23R is associated with CD and UC

A genome-wide association study testing over 300 000 autosomal SNPs was recently reported[120]. Three SNPs had nearly two orders of magnitude greater significance compared to the next most significant markers. Two of the three markers, were in the known CD susceptibility gene, Nod2. The third marker, rs11209026 ($P = 5.05 \times 10^{-9}$, corrected $P = 1.56 \times 10^{-3}$), was a non-synonymous SNP (Arg381Gln) in the *IL23R* gene on chromosome 1p31. Replication of these findings was observed in an independent cohort of 883 nuclear families and observed significant association in CD and non-Jewish UC. Therefore, *IL23R* represents both a CD and a UC susceptibility gene. The less common glutamine allele of Arg381Gln has an allele frequency of 1.9% in non-Jewish CD and 7.0% in non-Jewish controls and therefore protects against IBD[120]. Other variants within *IL23R* are also associated with IBD, independent of the Arg381Gln association. Since the initial report, these findings have subsequently been replicated in Scottish pediatric IBD[121] and Belgian CD[119] cohorts, indicating a clear role for *IL23R* in IBD susceptibility.

Given the IL-23 pathway's role in activation and perpetuation of organ-specific inflammatory responses, the genetic findings suggest that targeting the IL-23 pathway may be a rational therapeutic approach. In this regard, anti-p40 administration, which blocks both IL-23 and IL-12 activities, has proved promising[122]. 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 could potentially be exploited to define clinical outcomes. The genetic association and the pro-inflammatory role of IL-23 strongly prioritize this pathway as a therapeutic target in IBD.

The *IL23R* genetic association to IBD correlates precisely with recent immunologic advances in understanding of the IL-23 pathway. A number of murine models of colitis, including IL-10 deficiency, T cell mediated (CD45Rbhigh reconstituted in RAG deficiency) and non-T cell mediated (agonistic anti-CD40 RAG deficiency, H. hepaticus-induced) colitis demonstrated significant amelioration when crossed with p40 and p19-deficient mice. This demonstrates the requirement for an intact IL-23 pathway for a variety of intestinal inflammation pathways[123-126].

A complete understanding of the strongly protective effect of *IL23R* polymorphisms in IBD should not be solely restricted to pharmacologic approaches to mimic the Arg381Gln polymorphism. While it is logical to test anti-p19 antibodies in IBD[127], the underlying paradigm is one of an ongoing requirement for continued immune

suppression. However, it is unlikely that the approximately 14% of European ancestry individuals heterozygous for the glutamine allele harbor significant immunodeficiencies, and therefore these individuals provide a unique opportunity to better understand the lifelong, dynamic host-intestinal microbial interactions that are the key to IBD. We are born with a sterile intestine[25] and an immature immune system[128], with the rapid acquisition of intestinal flora evolving dynamically with early instruction signals to the host immune response. The significant susceptibility of neonates to infection may correlate with their incapacity to induce IL-12p35 with lipopolysaccharide stimulation[129], with IL-23p19 induction being largely intact[130]. This would suggest that functional IL-23 pathway polymorphisms may be particularly important in modulating neonatal development of intestinal tolerance and bacterial colonization.

ATG16L association to CD

In a genome-wide survey of nearly 20 000 nonsynonymous SNPs, an amino acid polymorphisms, Thr300Ala within the *ATG16L1* gene was found to be highly associated with CD. The *ATG16L1* protein is comprised of N-terminal APG16 domain consisting of coiled coils and eight C-terminal WD repeats. The Thr300Ala variant is located at the N-terminus of the WD-repeat domain in *ATG16L1*. The *ATG16L1* gene is part of the autophagosome pathway and has been implicated in the processing of intracellular bacteria. Of interest, no association was observed in UC and a statistical interaction was reported with the Nod2 (*CARD15*) CD associations. Since the initial report, this association has been confirmed in a separate, Belgian CD cohort[119]. In addition, in this cohort, no association was observed in UC. Taken together, the *ATG16L1* association represents a well-replicated CD-specific association.

Association of a gene desert on chromosome 5p13.1 which modulates the expression of the prostaglandin receptor EP4 (*PTGER4*)

In a genome-wide association study in a Belgian CD cohort, in addition to the *CARD15*, *IL23R* and *ATG16L1* associations, association was observed in a region on chromosome 5p13.1. The most significant association was observed in a gene desert region flanked by a number of potential candidate genes, including *CARD6*, complement factors C6, C7 and C9 and the prostaglandin receptor, EP4 (*PTGER4*). No association was observed in UC for markers in this region. *PTGER4* is a compelling candidate, in part because *PTGER4* deficient mice develop a more severe colitis with dextran sodium sulfate treatment[131]. In elegant analyses, the investigators compared SNP data in this genomic region with mRNA expression levels of flanking genes from the corresponding, individual lymphoblastoid cell lines. Throughout the region of association, a number of SNPs were significantly associated with mRNA expression levels of *PTGER4*, including at least one SNP demonstrating both CD association as well as correlation with *PTGER4* expression. However, the susceptibility allele at this marker corresponds with increased *PTGER4* expression, which is not consistent with the increased colitic susceptibility observed in *PTGER4*-deficient mice[131]. However, taken together, these findings strongly suggest that genetic variation in this region is associated with IBD and significantly regulates *PTGER4* expression.

Assessment of genome-wide association studies in IBD

It is anticipated that the most significant associations observed in various genome-wide association studies will be largely replicable between studies in comparable population cohorts. Genome-wide association studies provide a comprehensive, unbiased landscape of common variation contributing to disease. Three relative limitations to these studies should be noted, however. Apart from the highly significant, outlying associations such as those observed for the Nod2 and *IL23R* gene associations, many of the most biologically significant disease associations may confer less significant statistical associations, and be obscured by surrounding "noise" conferred by false positive associations. Study design approaches to dissect true disease associations from surrounding noise remains a major methodological challenge moving forward. A second limitation is that the genotyping platforms typically utilized for these studies are limited to testing common genetic variation, with minor allele frequencies of greater than 5%. In the presence of significant allelic heterogeneity for uncommon variants within a disease gene, genome-wide association approaches will be relatively insensitive. The Nod2 associations are observed in genome-wide surveys so clearly because all three of the uncommon CD variants by chance share a common haplotype background. Therefore, the identification and characterization of uncommon variants may largely be missed by a complete reliance of genome-wide association approaches. Finally, many of the early genome-wide genotyping platforms were specifically developed to efficiently test European ancestry cohorts, and may not as efficiently assay Asian or African populations. Particularly for African populations which have shorter regions of linkage disequilibrium, testing of larger numbers of genetic markers will be required.

From genetics to genetic epidemiology: defining risks in patient subsets to assist clinical practice

A more complete pathophysiologic genetic definition of IBD will require integration of clinical observations, genetic data, statistical analyses, and delineation of underlying biologic processes. Interrogating GWA data for gene-gene interactions analyses has been proposed as being more powerful than traditional, single locus analyses under a range of interactive models. However such analyses may be more powerful if prior biologic knowledge on signaling pathways, *in vivo* models of inflammation and integration of genetic insight is applied. The growing catalogue of functional polymorphisms contributing to distinct chronic inflammatory disorders, while not necessarily demonstrating disease association, may contribute to IBD pathophysiology as disease modifiers. Stratification of genetic associations by phenotype variation and/or use of covariates may benefit genetic understanding of IBD by reducing heterogeneity that can obscure association evidence. Because of the strength of the Nod2 association

in CD, and because ileal CD represents a large fraction of overall cases of CD, the Nod2 associations were easily observed in the "all CD" analyses, and even the "all IBD" analyses. However, as the Nod2 genotype risk represents one of the largest among complex disorders, it would be anticipated that many IBD association signals may require correct subsetting, of which disease location is most well-established. Integration of clinical and genotype data may provide the capacity to predict complications and disease course in a clinically useful way. While the integration of definitive IBD risk alleles would be the most straightforward factors to include in such predictive models, it is possible that many key genetic factors (particularly well-established functional polymorphisms from related disorders) may, by themselves, not be associated with the major phenotypes, but rather function solely as disease modifiers. For example, it is possible that certain key genetic factors (particularly well-established functional polymorphisms from related disorders) may, by themselves, not be associated with the major phenotypes but rather serve to modify disease expression. Detecting such relationships requires a conditional analysis restricted to those with IBD in which certain phenotypic characteristics are modeled as a function of the presence of one or more genetic variants. Such models will also provide a framework for generating predictions about clinical course among newly diagnosed cases-providing clinicians important information for use in treatment planning.

The identification of major single gene associations such as *IL23R* and Nod2 provides a framework around which risk models for multigenic disorders can be developed. A major research interest moving forward will be identifying gene-gene interactions that contribute to increased disease risk or define pathophysiological subsets within the disease. This will be explored through: (1) a broad-based, systematic search of genomic regions demonstrating the most significant evidence for association, (2) pathway analyses integrating specific knowledge regarding functional human polymorphisms relevant to the *IL23R* and Nod2 pathways, and (3) studying key mechanistic intermediates known to be particularly relevant to disease pathogenesis.

As functional genetic variants that predispose to IBD are identified, their effect on modifying disease phenotype and/or disease course will need to be examined. For example, the CARD15 mutations increase susceptibility to CD generally, affect disease location (increase susceptibility to ileal location), and modify disease behavior. Because of the challenges of longitudinal follow-up, prospective evaluation of IBD-associated mutations represents a major challenge. The efficient abstraction of critical research datapoints from active clinical practices in an accurate and reproducible manner will be required. Interpretation of disease course from multi-center studies given differences in practice patterns represents an additional interpretative challenge. However, such studies will provide the basis for improved translation of genetic discovery in IBD and will need to be designed *via* active communication and collaboration throughout the IBD research community.

REFERENCES

1 **Halfvarson J**, Bodin L, Tysk C, Lindberg E, Jarnerot G. Inflammatory bowel disease in a Swedish twin cohort: a long-term follow-up of concordance and clinical characteristics. *Gastroenterology* 2003; **124**: 1767-1773
2 **Orholm M**, Binder V, Sorensen TI, Rasmussen LP, Kyvik KO. Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. *Scand J Gastroenterol* 2000; **35**: 1075-1081
3 **Thompson NP**, Driscoll R, Pounder RE, Wakefield AJ. Genetics versus environment in inflammatory bowel disease: results of a British twin study. *BMJ* 1996; **312**: 95-96
4 **Tysk C**, Lindberg E, Jarnerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. *Gut* 1988; **29**: 990-996
5 **Binder V**. Genetic epidemiology in inflammatory bowel disease. *Dig Dis* 1998; **16**: 351-355
6 **Yang H**, Rotter JI. Genetic aspects of idiopathic inflammatory bowel disease. In: Kirsner JB, Shorter RG, editors. Inflammatory Bowel Disease. 4th ed. Baltimore: Williams & Wilkins, 1995: 301-331
7 **Yang H**, Rotter JI. Genetics of inflammatory bowel disease. In: Targan SR, Shanahan F, editors. Inflammatory bowel disease: from bench to bedside. Baltimore: Williams and Wilkins, 1994: 32-64
8 **Satsangi J**, Jewell DP, Bell JI. The genetics of inflammatory bowel disease. *Gut* 1997; **40**: 572-574
9 **Orholm M**, Munkholm P, Langholz E, Nielsen OH, Sorensen TI, Binder V. Familial occurrence of inflammatory bowel disease. *N Engl J Med* 1991; **324**: 84-88
10 **Loftus EV Jr**. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; **126**: 1504-1517
11 **Loftus EV Jr**, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. Crohn's disease in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gastroenterology* 1998; **114**: 1161-1168
12 **Loftus EV Jr**, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. Ulcerative colitis in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gut* 2000; **46**: 336-343
13 **Bernstein CN**, Blanchard JF, Rawsthorne P, Wajda A. Epidemiology of Crohn's disease and ulcerative colitis in a central Canadian province: a population-based study. *Am J Epidemiol* 1999; **149**: 916-924
14 **Sandler RS**, Targan SR, Shanahan F. Epidemiology. Inflammatory Bowel Disease: from bench to bedside. Baltimore MD: Williams & Wilkins, 1994: 5-30
15 **Calkins BM**, Mendelhoff AI. The epidemiology of idiopathic inflammatory bowel disease. In: Kirsner JB, Shorter RG, editors. Inflammatory Bowel Disease. 4th ed. Baltimore: Williams & Wilkins, 1995: 31-68
16 **Sonnenberg A**, McCarty DJ, Jacobsen SJ. Geographic variation of inflammatory bowel disease within the United States. *Gastroenterology* 1991; **100**: 143-149
17 **Sonnenberg A**, Wasserman IH. Epidemiology of inflammatory bowel disease among U.S. military veterans. *Gastroenterology* 1991; **101**: 122-130
18 **Kurata JH**, Kantor-Fish S, Frankl H, Godby P, Vadheim CM. Crohn's disease among ethnic groups in a large health maintenance organization. *Gastroenterology* 1992; **102**: 1940-1948
19 **Hiatt RA**, Kaufman L. Epidemiology of inflammatory bowel disease in a defined northern California population. *West J Med* 1988; **149**: 541-546
20 **Ogunbi SO**, Ransom JA, Sullivan K, Schoen BT, Gold BD. Inflammatory bowel disease in African-American children living in Georgia. *J Pediatr* 1998; **133**: 103-107
21 **Yang H**, Rotter JI, Targan SR, Shanahan F. Genetics of inflammatory bowel disease. Inflammatory Bowel Disease: from bench to bedside. Baltimore MD: Williams & Wilkins,

1994: 32-64
22 **Gent AE**, Hellier MD, Grace RH, Swarbrick ET, Coggon D. Inflammatory bowel disease and domestic hygiene in infancy. *Lancet* 1994; **343**: 766-767
23 **Bernstein CN**, Rawsthorne P, Cheang M, Blanchard JF. A population-based case control study of potential risk factors for IBD. *Am J Gastroenterol* 2006; **101**: 993-1002
24 **Amre DK**, Lambrette P, Law L, Krupoves A, Chotard V, Costea F, Grimard G, Israel D, Mack D, Seidman EG. Investigating the hygiene hypothesis as a risk factor in pediatric onset Crohn's disease: a case-control study. *Am J Gastroenterol* 2006; **101**: 1005-1011
25 **Ley RE**, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 2006; **124**: 837-848
26 **Guarner F**, Bourdet-Sicard R, Brandtzaeg P, Gill HS, McGuirk P, van Eden W, Versalovic J, Weinstock JV, Rook GA. Mechanisms of disease: the hygiene hypothesis revisited. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 275-284
27 **Kalliomaki M**, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; **357**: 1076-1079
28 **Lindberg E**, Tysk C, Andersson K, Jarnerot G. Smoking and inflammatory bowel disease. A case control study. *Gut* 1988; **29**: 352-357
29 **Boyko EJ**, Koepsell TD, Perera DR, Inui TS. Risk of ulcerative colitis among former and current cigarette smokers. *N Engl J Med* 1987; **316**: 707-710
30 **Tuvlin JA**, Raza SS, Bracamonte S, Julian C, Hanauer SB, Nicolae DL, King AC, Cho JH. Smoking and inflammatory bowel disease: trends in familial and sporadic cohorts. *Inflamm Bowel Dis* 2007; **13**: 573-579
31 **Bridger S**, Lee JC, Bjarnason I, Jones JE, Macpherson AJ. In siblings with similar genetic susceptibility for inflammatory bowel disease, smokers tend to develop Crohn's disease and non-smokers develop ulcerative colitis. *Gut* 2002; **51**: 21-25
32 **Sutherland LR**, Ramcharan S, Bryant H, Fick G. Effect of cigarette smoking on recurrence of Crohn's disease. *Gastroenterology* 1990; **98**: 1123-1128
33 **Anttila S**, Hakkola J, Tuominen P, Elovaara E, Husgafvel-Pursiainen K, Karjalainen A, Hirvonen A, Nurminen T. Methylation of cytochrome P4501A1 promoter in the lung is associated with tobacco smoking. *Cancer Res* 2003; **63**: 8623-8628
34 **Reich DE**, Gabriel SB, Altshuler D. Quality and completeness of SNP databases. *Nat Genet* 2003; **33**: 457-458
35 **The International HapMap Project**. *Nature* 2003; **426**: 789-796
36 **Pe'er I**, de Bakker PI, Maller J, Yelensky R, Altshuler D, Daly MJ. Evaluating and improving power in whole-genome association studies using fixed marker sets. *Nat Genet* 2006; **38**: 663-667
37 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nunez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606
38 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603
39 **Bonen DK**, Ogura Y, Nicolae DL, Inohara N, Saab L, Tanabe T, Chen FF, Foster SJ, Duerr RH, Brant SR, Cho JH, Nunez G. Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. *Gastroenterology* 2003; **124**: 140-106
40 **Girardin SE**, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem* 2003; **278**: 8869-8872
41 **Inohara N**, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, Fukase K, Inamura S, Kusumoto S, Hashimoto M, Foster SJ, Moran AP, Fernandez-Luna JL, Nunez G. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* 2003; **278**: 5509-5512
42 **Kobayashi KS**, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, Flavell RA. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 2005; **307**: 731-734
43 **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**: 2393-2404
44 **Bonen DK**, Ogura Y, Nicolae DL, Inohara N, Saab L, Tanabe T, Chen FF, Foster SJ, Duerr RH, Brant SR, Cho JH, Nunez G. Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. *Gastroenterology* 2003; **124**: 140-146
45 **The ENCODE (ENCyclopedia Of DNA Elements) Project**. *Science* 2004; **306**: 636-640
46 **Netea MG**, Ferwerda G, de Jong DJ, Jansen T, Jacobs L, Kramer M, Naber TH, Drenth JP, Girardin SE, Kullberg BJ, Adema GJ, Van der Meer JW. Nucleotide-binding oligomerization domain-2 modulates specific TLR pathways for the induction of cytokine release. *J Immunol* 2005; **174**: 6518-6523
47 **McGovern DP**, Hysi P, Ahmad T, van Heel DA, Moffatt MF, Carey A, Cookson WO, Jewell DP. Association between a complex insertion/deletion polymorphism in NOD1 (CARD4) and susceptibility to inflammatory bowel disease. *Hum Mol Genet* 2005; **14**: 1245-1250
48 **Kramer M**, Netea MG, de Jong DJ, Kullberg BJ, Adema GJ. Impaired dendritic cell function in Crohn's disease patients with NOD2 3020insC mutation. *J Leukoc Biol* 2006; **79**: 860-866
49 **Yamazaki K**, Takazoe M, Tanaka T, Kazumori T, Nakamura Y. Absence of mutation in the NOD2/CARD15 gene among 483 Japanese patients with Crohn's disease. *J Hum Genet* 2002; **47**: 469-472
50 **Croucher PJ**, Mascheretti S, Hampe J, Huse K, Frenzel H, Stoll M, Lu T, Nikolaus S, Yang SK, Krawczak M, Kim WH, Schreiber S. Haplotype structure and association to Crohn's disease of CARD15 mutations in two ethnically divergent populations. *Eur J Hum Genet* 2003; **11**: 6-16
51 **Leong RW**, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP, Sung JJ. NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population. *Aliment Pharmacol Ther* 2003; **17**: 1465-1470
52 **Kugathasan S**, Loizides A, Babusukumar U, McGuire E, Wang T, Hooper P, Nebel J, Kofman G, Noel R, Broeckel U, Tolia V. Comparative phenotypic and CARD15 mutational analysis among African American, Hispanic, and White children with Crohn's disease. *Inflamm Bowel Dis* 2005; **11**: 631-638
53 **Pauleau AL**, Murray PJ. Role of nod2 in the response of macrophages to toll-like receptor agonists. *Mol Cell Biol* 2003; **23**: 7531-7539
54 **Lala S**, Ogura Y, Osborne C, Hor SY, Bromfield A, Davies S, Ogunbiyi O, Nunez G, Keshav S. Crohn's disease and the NOD2 gene: a role for paneth cells. *Gastroenterology* 2003; **125**: 47-57
55 **Ogura Y**, Lala S, Xin W, Smith E, Dowds TA, Chen FF, Zimmermann E, Tretiakova M, Cho JH, Hart J, Greenson JK, Keshav S, Nunez G. Expression of NOD2 in Paneth cells: a possible link to Crohn's ileitis. *Gut* 2003; **52**: 1591-1597
56 **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**: 2393-2404
57 **Kobayashi KS**, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nunez G, Flavell RA. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 2005; **307**: 731-734
58 **Wehkamp J**, Harder J, Weichenthal M, Schwab M, Schaffeler E, Schlee M, Herrlinger KR, Stallmach A, Noack F, Fritz P, Schroder JM, Bevins CL, Fellermann K, Stange EF. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut* 2004; **53**: 1658-1664
59 **Wehkamp J**, Salzman NH, Porter E, Nuding S, Weichenthal M, Petras RE, Shen B, Schaeffeler E, Schwab M, Linzmeier R,

Feathers RW, Chu H, Lima H Jr, Fellermann K, Ganz T, Stange EF, Bevins CL. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. *Proc Natl Acad Sci USA* 2005; **102**: 18129-18134

60 **Rioux JD**, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 2001; **29**: 223-228

61 **Armuzzi A**, Ahmad T, Ling KL, de Silva A, Cullen S, van Heel D, Orchard TR, Welsh KI, Marshall SE, Jewell DP. Genotype-phenotype analysis of the Crohn's disease susceptibility haplotype on chromosome 5q31. *Gut* 2003; **52**: 1133-1139

62 **Giallourakis C**, Stoll M, Miller K, Hampe J, Lander ES, Daly MJ, Schreiber S, Rioux JD. IBD5 is a general risk factor for inflammatory bowel disease: replication of association with Crohn disease and identification of a novel association with ulcerative colitis. *Am J Hum Genet* 2003; **73**: 205-211

63 **Mirza MM**, Fisher SA, King K, Cuthbert AP, Hampe J, Sanderson J, Mansfield J, Donaldson P, Macpherson AJ, Forbes A, Schreiber S, Lewis CM, Mathew CG. Genetic evidence for interaction of the 5q31 cytokine locus and the CARD15 gene in Crohn disease. *Am J Hum Genet* 2003; **72**: 1018-1022

64 **Negoro K**, McGovern DP, Kinouchi Y, Takahashi S, Lench NJ, Shimosegawa T, Carey A, Cardon LR, Jewell DP, van Heel DA. Analysis of the IBD5 locus and potential gene-gene interactions in Crohn's disease. *Gut* 2003; **52**: 541-546

65 **Waller S**, Tremelling M, Bredin F, Godfrey L, Howson J, Parkes M. Evidence for association of OCTN genes and IBD5 with ulcerative colitis. *Gut* 2006; **55**: 809-814

66 **Peltekova VD**, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH, Siminovitch KA. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004; **36**: 471-475

67 **Fisher SA**, Hampe J, Onnie CM, Daly MJ, Curley C, Purcell S, Sanderson J, Mansfield J, Annese V, Forbes A, Lewis CM, Schreiber S, Rioux JD, Mathew CG. Direct or indirect association in a complex disease: the role of SLC22A4 and SLC22A5 functional variants in Crohn disease. *Hum Mutat* 2006; **27**: 778-785

68 **Noble CL**, Nimmo ER, Drummond H, Ho GT, Tenesa A, Smith L, Anderson N, Arnott ID, Satsangi J. The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease. *Gastroenterology* 2005; **129**: 1854-1864

69 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus Jr EV, Pena AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5-36

70 **Torok HP**, Glas J, Tonenchi L, Lohse P, Muller-Myhsok B, Limbersky O, Neugebauer C, Schnitzler F, Seiderer J, Tillack C, Brand S, Brunnler G, Jagiello P, Epplen JT, Griga T, Klein W, Schiemann U, Folwaczny M, Ochsenkuhn T, Folwaczny C. Polymorphisms in the DLG5 and OCTN cation transporter genes in Crohn's disease. *Gut* 2005; **54**: 1421-1427

71 **Palmieri O**, Latiano A, Valvano R, D'Inca R, Vecchi M, Sturniolo GC, Saibeni S, Peyvandi F, Bossa F, Zagaria C, Andriulli A, Devoto M, Annese V. Variants of OCTN1-2 cation transporter genes are associated with both Crohn's disease and ulcerative colitis. *Aliment Pharmacol Ther* 2006; **23**: 497-506

72 **Latiano A**, Palmieri O, Valvano RM, D'Inca R, Vecchi M, Ferraris A, Sturniolo GC, Spina L, Lombardi G, Dallapiccola B, Andriulli A, Devoto M, Annese V. Contribution of IBD5 locus to clinical features of IBD patients. *Am J Gastroenterol* 2006; **101**: 318-325

73 **Russell RK**, Drummond HE, Nimmo ER, Anderson NH, Noble CL, Wilson DC, Gillett PM, McGrogan P, Hassan K, Weaver LT, Bisset WM, Mahdi G, Satsangi J. Analysis of the influence of OCTN1/2 variants within the IBD5 locus on disease susceptibility and growth indices in early onset inflammatory bowel disease. *Gut* 2006; **55**: 1114-1123

74 **Babusukumar** U, Wang T, McGuire E, Broeckel U, Kugathasan S. Contribution of OCTN variants within the IBD5 locus to pediatric onset Crohn's disease. *Am J Gastroenterol* 2006; **101**: 1354-1361

75 **Silverberg MS**, Duerr RH, Brant SR, Bromfield G, Datta LW, Jani N, Kane SV, Rotter JI, Philip Schumm L, Hillary Steinhart A, Taylor KD, Yang H, Cho JH, Rioux JD, Daly MJ. Refined genomic localization and ethnic differences observed for the IBD5 association with Crohn's disease. *Eur J Hum Genet* 2007; **15**: 328-335

76 **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**: 1080-1084

77 **Trachtenberg EA**, Yang H, Hayes E, Vinson M, Lin C, Targan SR, Tyan D, Erlich H, Rotter JI. HLA class II haplotype associations with inflammatory bowel disease in Jewish (Ashkenazi) and non-Jewish caucasian populations. *Hum Immunol* 2000; **61**: 326-333

78 **Silverberg MS**, Mirea L, Bull SB, Murphy JE, Steinhart AH, Greenberg GR, McLeod RS, Cohen Z, Wade JA, Siminovitch KA. 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

79 **Karban AS**, Okazaki T, Panhuysen CI, Gallegos T, Potter JJ, Bailey-Wilson JE, Silverberg MS, Duerr RH, Cho JH, Gregersen PK, Wu Y, Achkar JP, Dassopoulos T, Mezey E, Bayless TM, Nouvet FJ, Brant SR. Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. *Hum Mol Genet* 2004; **13**: 35-45

80 **Borm ME**, van Bodegraven AA, Mulder CJ, Kraal G, Bouma G. A NFKB1 promoter polymorphism is involved in susceptibility to ulcerative colitis. *Int J Immunogenet* 2005; **32**: 401-405

81 **Oliver J**, Gomez-Garcia M, Paco L, Lopez-Nevot MA, Pinero A, Correro F, Martin L, Brieva JA, Nieto A, Martin J. A functional polymorphism of the NFKB1 promoter is not associated with ulcerative colitis in a Spanish population. *Inflamm Bowel Dis* 2005; **11**: 576-579

82 **Glas J**, Torok HP, Tonenchi L, Muller-Myhsok B, Mussack T, Wetzke M, Klein W, Epplen JT, Griga T, Schiemann U, Lohse P, Seiderer J, Schnitzler F, Brand S, Ochsenkuhn T, Folwaczny M, Folwaczny C. Role of the NFKB1 -94ins/delATTG promoter polymorphism in IBD and potential interactions with polymorphisms in the CARD15/NOD2, IKBL, and IL-1RN genes. *Inflamm Bowel Dis* 2006; **12**: 606-611

83 **Schwab M**, Schaeffeler E, Marx C, Fromm MF, Kaskas B, Metzler J, Stange E, Herfarth H, Schoelmerich J, Gregor M, Walker S, Cascorbi I, Roots I, Brinkmann U, Zanger UM, Eichelbaum M. Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. *Gastroenterology* 2003; **124**: 26-33

84 **Brant SR**, Panhuysen CI, Nicolae D, Reddy DM, Bonen DK, Karaliukas R, Zhang L, Swanson E, Datta LW, Moran T, Ravenhill G, Duerr RH, Achkar JP, Karban AS, Cho JH. MDR1 Ala893 polymorphism is associated with inflammatory bowel disease. *Am J Hum Genet* 2003; **73**: 1282-1292

85 **Croucher PJ**, Mascheretti S, Foelsch UR, Hampe J, Schreiber S. Lack of association between the C3435T MDR1 gene polymorphism and inflammatory bowel disease in two independent Northern European populations. *Gastroenterology* 2003; **125**: 1919-1920; author reply 1920-1921

86 **Gazouli M**, Zacharatos P, Gorgoulis V, Mantzaris G, Papalambros E, Ikonomopoulos J. The C3435T MDR1 gene polymorphism is not associated with susceptibility for ulcerative colitis in Greek population. *Gastroenterology* 2004; **126**: 367-369

87 **Glas J**, Torok HP, Schiemann U, Folwaczny C. MDR1 gene polymorphism in ulcerative colitis. *Gastroenterology* 2004; **126**: 367

88 **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

89 **Ho GT**, Nimmo ER, Tenesa A, Fennell J, Drummond H, Mowat C, Arnott ID, Satsangi J. Allelic variations of the multidrug resistance gene determine susceptibility and disease behavior in ulcerative colitis. *Gastroenterology* 2005; **128**: 288-296

90 **Ho GT**, Soranzo N, Nimmo ER, Tenesa A, Goldstein DB, Satsangi J. ABCB1/MDR1 gene determines susceptibility and phenotype in ulcerative colitis: discrimination of critical variants using a gene-wide haplotype tagging approach. *Hum Mol Genet* 2006; **15**: 797-805

91 **Urcelay E**, Mendoza JL, Martin MC, Mas A, Martinez A, Taxonera C, Fernandez-Arquero M, Diaz-Rubio M, de la Concha EG. MDR1 gene: susceptibility in Spanish Crohn's disease and ulcerative colitis patients. *Inflamm Bowel Dis* 2006; **12**: 33-37

92 **Oostenbrug LE**, Dijkstra G, Nolte IM, van Dullemen HM, Oosterom E, Faber KN, de Jong DJ, van der Linde K, te Meerman GJ, van der Steege G, Kleibeuker JH, Jansen PL. Absence of association between the multidrug resistance (MDR1) gene and inflammatory bowel disease. *Scand J Gastroenterol* 2006; **41**: 1174-1182

93 **Stoll M**, Corneliussen B, Costello CM, Waetzig GH, Mellgard B, Koch WA, Rosenstiel P, Albrecht M, Croucher PJ, Seegert D, Nikolaus S, Hampe J, Lengauer T, Pierrou S, Foelsch UR, Mathew CG, Lagerstrom-Fermer M, Schreiber S. Genetic variation in DLG5 is associated with inflammatory bowel disease. *Nat Genet* 2004; **36**: 476-480

94 **Daly MJ**, Pearce AV, Farwell L, Fisher SA, Latiano A, Prescott NJ, Forbes A, Mansfield J, Sanderson J, Langelier D, Cohen A, Bitton A, Wild G, Lewis CM, Annese V, Mathew CG, Rioux JD. Association of DLG5 R30Q variant with inflammatory bowel disease. *Eur J Hum Genet* 2005; **13**: 835-839

95 **Noble CL**, Nimmo ER, Drummond H, Smith L, Arnott ID, Satsangi J. DLG5 variants do not influence susceptibility to inflammatory bowel disease in the Scottish population. *Gut* 2005; **54**: 1416-1420

96 **Friedrichs F**, Brescianini S, Annese V, Latiano A, Berger K, Kugathasan S, Broeckel U, Nikolaus S, Daly MJ, Schreiber S, Rioux JD, Stoll M. Evidence of transmission ratio distortion of DLG5 R30Q variant in general and implication of an association with Crohn disease in men. *Hum Genet* 2006; **119**: 305-311

97 **Newman WG**, Gu X, Wintle RF, Liu X, van Oene M, Amos CI, Siminovitch KA. DLG5 variants contribute to Crohn disease risk in a Canadian population. *Hum Mutat* 2006; **27**: 353-358

98 **Medici V,** Mascheretti S, Croucher PJ, Stoll M, Hampe J, Grebe J, Sturniolo GC, Solberg C, Jahnsen J, Moum B, Schreiber S, Vatn MH. Extreme heterogeneity in CARD15 and DLG5 Crohn disease-associated polymorphisms between German and Norwegian populations. *Eur J Hum Genet* 2006; **14**: 459-468

99 **Buning C**, Geerdts L, Fiedler T, Gentz E, Pitre G, Reuter W, Luck W, Buhner S, Molnar T, Nagy F, Lonovics J, Dignass A, Landt O, Nickel R, Genschel J, Lochs H, Schmidt HH, Witt H. DLG5 variants in inflammatory bowel disease. *Am J Gastroenterol* 2006; **101**: 786-792

100 **Tremelling M**, Waller S, Bredin F, Greenfield S, Parkes M. Genetic variants in TNF-alpha but not DLG5 are associated with inflammatory bowel disease in a large United Kingdom cohort. *Inflamm Bowel Dis* 2006; **12**: 178-184

101 **Lakatos PL**, Fischer S, Claes K, Kovacs A, Molnar T, Altorjay I, Demeter P, Tulassay Z, Palatka K, Papp M, Rutgeerts P, Szalay F, Papp J, Vermeire S, Lakatos L. DLG5 R30Q is not associated with IBD in Hungarian IBD patients but predicts clinical response to steroids in Crohn's disease. *Inflamm Bowel Dis* 2006; **12**: 362-368

102 **Pearce AV**, Fisher SA, Prescott NJ, Onnie CM, Pattni R, Green P, Forbes A, Mansfield J, Sanderson J, Schreiber S, Lewis CM, Mathew CG. Investigation of association of the DLG5 gene with phenotypes of inflammatory bowel disease in the British population. *Int J Colorectal Dis* 2007; **22**: 419-424

103 **Zouali H**, Lesage S, Merlin F, Cezard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Christensen S, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Chamaillard M, Thomas G, Hugot JP. CARD4/NOD1 is not involved in inflammatory bowel disease. *Gut* 2003; **52**: 71-74

104 **Tremelling M**, Hancock L, Bredin F, Sharpstone D, Bingham SA, Parkes M. Complex insertion/deletion polymorphism in NOD1 (CARD4) is not associated with inflammatory bowel disease susceptibility in East Anglia panel. *Inflamm Bowel Dis* 2006; **12**: 967-971

105 **Franke A**, Ruether A, Wedemeyer N, Karlsen TH, Nebel A, Schreiber S. No association between the functional CARD4 insertion/deletion polymorphism and inflammatory bowel diseases in the German population. *Gut* 2006; **55**: 1679-1680

106 **Carter MJ**, di Giovine FS, Jones S, Mee J, Camp NJ, Lobo AJ, Duff GW. Association of the interleukin 1 receptor antagonist gene with ulcerative colitis in Northern European Caucasians. *Gut* 2001; **48**: 461-467

107 **Arnott ID**, Nimmo ER, Drummond HE, Fennell J, Smith BR, MacKinlay E, Morecroft J, Anderson N, Kelleher D, O'Sullivan M, McManus R, Satsangi J. NOD2/CARD15, TLR4 and CD14 mutations in Scottish and Irish Crohn's disease patients: evidence for genetic heterogeneity within Europe? *Genes Immun* 2004; **5**: 417-425

108 **Franchimont D**, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, Quertinmont E, Abramowicz M, Van Gossum A, Deviere J, Rutgeerts P. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 2004; **53**: 987-992

109 **Torok HP**, Glas J, Tonenchi L, Mussack T, Folwaczny C. Polymorphisms of the lipopolysaccharide-signaling complex in inflammatory bowel disease: association of a mutation in the Toll-like receptor 4 gene with ulcerative colitis. *Clin Immunol* 2004; **112**: 85-91

110 **Gazouli M**, Mantzaris G, Kotsinas A, Zacharatos P, Papalambros E, Archimandritis A, Ikonomopoulos J, Gorgoulis VG. Association between polymorphisms in the Toll-like receptor 4, CD14, and CARD15/NOD2 and inflammatory bowel disease in the Greek population. *World J Gastroenterol* 2005; **11**: 681-685

111 **Lakatos PL**, Lakatos L, Szalay F, Willheim-Polli C, Osterreicher C, Tulassay Z, Molnar T, Reinisch W, Papp J, Mozsik G, Ferenci P. Toll-like receptor 4 and NOD2/CARD15 mutations in Hungarian patients with Crohn's disease: phenotype-genotype correlations. *World J Gastroenterol* 2005; **11**: 1489-1495

112 **Oostenbrug LE**, Drenth JP, de Jong DJ, Nolte IM, Oosterom E, van Dullemen HM, van der Linde K, te Meerman GJ, van der Steege G, Kleibeuker JH, Jansen PL. Association between Toll-like receptor 4 and inflammatory bowel disease. *Inflamm Bowel Dis* 2005; **11**: 567-575

113 **Ouburg S**, Mallant-Hent R, Crusius JB, van Bodegraven AA, Mulder CJ, Linskens R, Pena AS, Morre SA. The toll-like receptor 4 (TLR4) Asp299Gly polymorphism is associated with colonic localisation of Crohn's disease without a major role for the Saccharomyces cerevisiae mannan-LBP-CD14-TLR4 pathway. *Gut* 2005; **54**: 439-440

114 **van Bodegraven AA**, Curley CR, Hunt KA, Monsuur AJ, Linskens RK, Onnie CM, Crusius JB, Annese V, Latiano A, Silverberg MS, Bitton A, Fisher SA, Steinhart AH, Forbes A, Sanderson J, Prescott NJ, Strachan DP, Playford RJ, Mathew CG, Wijmenga C, Daly MJ, Rioux JD, van Heel DA. Genetic variation in myosin IXB is associated with ulcerative colitis. *Gastroenterology* 2006; **131**: 1768-1774

115 **Monsuur AJ**, de Bakker PI, Alizadeh BZ, Zhernakova A, Bevova MR, Strengman E, Franke L, van't Slot R, van Belzen MJ, Lavrijsen IC, Diosdado B, Daly MJ, Mulder CJ, Mearin ML, Meijer JW, Meijer GA, van Oort E, Wapenaar MC, Koeleman BP, Wijmenga C. Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect. *Nat Genet* 2005; **37**: 1341-1344

116 **Yamazaki K**, McGovern D, Ragoussis J, Paolucci M, Butler H, Jewell D, Cardon L, Takazoe M, Tanaka T, Ichimori T, Saito S, Sekine A, Iida A, Takahashi A, Tsunoda T, Lathrop M, Nakamura Y. Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. *Hum Mol Genet* 2005; **14**: 3499-3506
117 **McGovern DP**, Butler H, Ahmad T, Paolucci M, van Heel DA, Negoro K, Hysi P, Ragoussis J, Travis SP, Cardon LR, Jewell DP. TUCAN (CARD8) genetic variants and inflammatory bowel disease. *Gastroenterology* 2006; **131**: 1190-1196
118 **Bamias G**, Martin C 3rd, Marini M, Hoang S, Mishina M, Ross WG, Sachedina MA, Friel CM, Mize J, Bickston SJ, Pizarro TT, Wei P, Cominelli F. Expression, localization, and functional activity of TL1A, a novel Th1-polarizing cytokine in inflammatory bowel disease. *J Immunol* 2003; **171**: 4868-4874
119 **Libioulle C**, Louis E, Hansoul S, Sandor C, Farnir F, Franchimont D, Vermeire S, Dewit O, de Vos M, Dixon A, Demarche B, Gut I, Heath S, Foglio M, Liang L, Laukens D, Mni M, Zelenika D, Van Gossum A, Rutgeerts P, Belaiche J, Lathrop M, Georges M. 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
120 **Duerr RH**, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006; **314**: 1461-1463
121 **Van Limbergen J**, Russell RK, Nimmo ER, Drummond HE, Smith L, Davies G, Anderson NH, Gillett PM, McGrogan P, Hassan K, Weaver L, Bisset WM, Mahdi G, Wilson DC, Satsangi J. IL23R Arg381Gln is associated with childhood onset inflammatory bowel disease in Scotland. *Gut* 2007; **56**: 1173-1174
122 **Mannon PJ**, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, Dolin B, Goodman N, Groden C, Hornung RL, Quezado M, Yang Z, Neurath MF, Salfeld J, Veldman GM, Schwertschlag U, Strober W. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med* 2004; **351**: 2069-2079
123 **Yen D**, Cheung J, Scheerens H, Poulet F, McClanahan T, McKenzie B, Kleinschek MA, Owyang A, Mattson J, Blumenschein W, Murphy E, Sathe M, Cua DJ, Kastelein RA, Rennick D. IL-23 is essential for T cell-mediated colitis and promotes inflammation via IL-17 and IL-6. *J Clin Invest* 2006; **116**: 1310-1316
124 **Hue S**, Ahern P, Buonocore S, Kullberg MC, Cua DJ, McKenzie BS, Powrie F, Maloy KJ. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J Exp Med* 2006; **203**: 2473-2483
125 **Kullberg MC**, Jankovic D, Feng CG, Hue S, Gorelick PL, McKenzie BS, Cua DJ, Powrie F, Cheever AW, Maloy KJ, Sher A. IL-23 plays a key role in Helicobacter hepaticus-induced T cell-dependent colitis. *J Exp Med* 2006; **203**: 2485-2494
126 **Uhlig HH**, McKenzie BS, Hue S, Thompson C, Joyce-Shaikh B, Stepankova R, Robinson N, Buonocore S, Tlaskalova-Hogenova H, Cua DJ, Powrie F. Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. *Immunity* 2006; **25**: 309-318
127 **Bowman EP**, Chackerian AA, Cua DJ. Rationale and safety of anti-interleukin-23 and anti-interleukin-17A therapy. *Curr Opin Infect Dis* 2006; **19**: 245-252
128 **Velilla PA**, Rugeles MT, Chougnet CA. Defective antigen-presenting cell function in human neonates. *Clin Immunol* 2006; **121**: 251-259
129 **Goriely S**, Vincart B, Stordeur P, Vekemans J, Willems F, Goldman M, De Wit D. Deficient IL-12(p35) gene expression by dendritic cells derived from neonatal monocytes. *J Immunol* 2001; **166**: 2141-2146
130 **Vanden Eijnden S**, Goriely S, De Wit D, Goldman M, Willems F. Preferential production of the IL-12(p40)/IL-23(p19) heterodimer by dendritic cells from human newborns. *Eur J Immunol* 2006; **36**: 21-26
131 **Kabashima K**, Saji T, Murata T, Nagamachi M, Matsuoka T, Segi E, Tsuboi K, Sugimoto Y, Kobayashi T, Miyachi Y, Ichikawa A, Narumiya S. The prostaglandin receptor EP4 suppresses colitis, mucosal damage and CD4 cell activation in the gut. *J Clin Invest* 2002; **109**: 883-893

S- Editor Liu Y **E- Editor** Liu Y