

# Genetics of inflammatory bowel disease: Current status and future directions

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The genetic analysis of complex diseases has become a mainstream biomedical research goal. The methods for genetically dissecting human diseases have now evolved into an integrated science combining human genetics, functional genomics, high-throughput experimental technology and computational techniques. The inflammatory bowel diseases (IBDs) feature prominently in this field, and the realm of IBD genetics is continually and exponentially increasing. The present review will summarize and put into perspective the breadth of IBD genetics as it has evolved over the past 10 years, and will give the reader some sense of anticipation as to where it may be leading us.

## EPIDEMIOLOGY – EVIDENCE FOR A GENETIC ETIOLOGY

A variety of epidemiological data suggest that genetic factors are intimately involved in the pathogenesis of IBD. For instance, IBD demonstrates a familial pattern of disease with a much higher disease frequency in first-degree relatives of affected individuals compared with the general population (1-4). The degree of 'heritability' or  $\lambda_s$  (the relative risk of the sibling of an affected individual) is estimated to range from 15 to 35 for Crohn's disease (CD) and six to nine for ulcerative colitis (UC) (5-9). Twin studies strengthen the argument for a genetic basis for IBD, with a much higher rate of disease concordance demonstrated in monozygotic twins compared with dizygotic twins, particularly in CD (10-13). Marked ethnic and racial differences in disease prevalence have been noted, particularly in Ashkenazi Jews, and are also in keeping with genetic etiology (14-22).

IBD is said to be a complex genetic disorder with the disease appearing to occur in susceptible individuals carrying one or more risk alleles after exposure to one or more environmental triggers. Because there is an increased risk of UC in CD-affected families and vice versa, a genetic model is proposed in which CD and UC are related diseases sharing some susceptibility loci but perhaps differing at others, which results in diverging disease courses (23,24). Additional genetic 'hits' or environmental factors may determine the particular phenotype of the individual by influencing features such as disease site, disease behaviour or response to therapy, among others (Figure 1).

## GENE IDENTIFICATION TECHNIQUES

The quest for IBD susceptibility genes has used two broad approaches: linkage analysis using multiple-affected families and association using affected individuals in a case-control

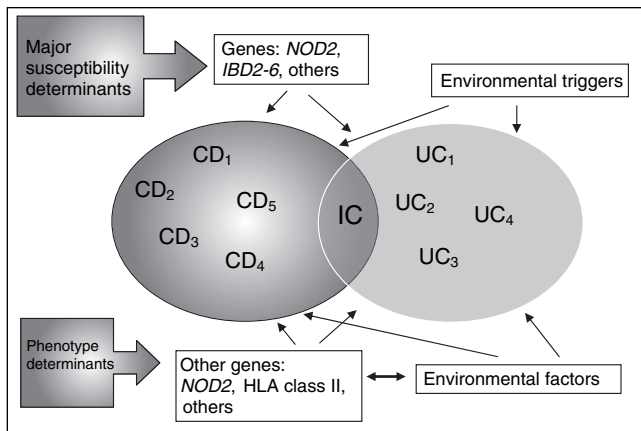
format. Genome-wide scans (GWSs) were traditionally used only with linkage analysis but recent progress has allowed a genome-wide approach by case-control association studies. Given the almost infinite number of possible genetic candidates, it is not surprising that the first major advances in this area were generated by linkage analysis: a 'hypothesis-free' technique in which the entire genome can be evaluated to look for areas of genetic susceptibility before trying to narrow down the search to specific genes. A genome-wide linkage analysis usually requires the ascertainment of numerous 'sibling pair' families, that is, two affected siblings and their parents (whether affected or not). Genotyping of all the members of each family is performed for polymorphic DNA microsatellite markers (approximately 300 microsatellite markers), which are located at intervals throughout the genome. Linkage analysis generates log of odds (LOD) scores which are measures of excess sharing of the same allele between affected siblings, suggesting a correlation between inheritance of disease and inheritance of that particular allele (Figure 1). This allows for the narrowing down of the search for susceptibility genes to a more specific part of the genome. Numerous genome-wide linkage studies in IBD have been published since 1996, identifying at least nine susceptibility loci (*IBD1* – *IBD9*) thought to contain causal genes (Table 1).

On the other hand, candidate gene analysis to some extent is hypothesis driven. It is an 'association test' that examines differences in the allele frequencies of the nominated gene in affected patients compared with controls. A variety of different statistical techniques can be used and the controls can be 'population-based' controls who must be carefully matched, or family-based controls who are often the parents of the affected individual. The chosen 'candidate' is usually based on findings from an earlier linkage study putting that gene within the susceptibility region identified in a prior GWS (a positional candidate) or by the purported function of the gene such as tumour necrosis factor-alpha (*TNF- $\alpha$* ) (a functional candidate). These approaches are certainly complementary, and ideally, both approaches are required in tandem, as was illustrated with the identification of the first IBD susceptibility gene described below. More recently, single nucleotide polymorphisms (SNPs) have been used in the place of microsatellite markers for the purpose of performing genome-wide association studies. SNPs represent sites along the genome in which a single base pair variation occurs from person to person where the least frequent allele has a frequency of 1% or greater. Many SNPs occur within genes, with some representing variations that alter gene function and may even represent the genetic lesion of interest.

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**Figure 1** Gene-environment interactions in inflammatory bowel disease (IBD). CD Crohn's disease; HLA Human leukocyte antigen; IC Indeterminate colitis; NOD2 Nucleotide oligomerization domain 2; UC Ulcerative colitis

Genome-wide association studies may represent the most powerful approach to identifying all of the IBD susceptibility genes that are relevant to disease pathophysiology because as many as 500,000 SNPs could be assayed for each patient in the study, making a massive amount of data available for analysis.

### CARD15/NOD2 AND CD SUSCEPTIBILITY

In 2001, two groups identified the first gene contributing to CD susceptibility within the *IBD1* susceptibility locus on chromosome 16. This gene was known as caspase activating recruitment domain 15 (*CARD15*) or nucleotide-binding oligomerization domain 2 (*NOD2*) (25,26), and is the only confirmed susceptibility gene to date for IBD. Three major independent polymorphisms of this gene are associated with CD in Caucasians: Arg702Trp and Gly908Arg are both missense mutations resulting in an amino acid substitution, as well as Leu1007fsinsC, which is a frameshift mutation that results in shortening of the protein product (25,27-43). While the identified mutations are either rare or absent in Asian (32,33,44-46), Arab (47,48) and African (49) populations, it is estimated that 27% to 38% of Caucasian CD patients carry one of the major risk alleles (compared with approximately 20% of Caucasian controls) and an additional 8% to 17% carry two copies (compared with less than 1% of controls). Of note, allele frequency for the three common mutations in sporadic CD is comparable with that seen in familial CD. A recent meta-analysis (50) calculated that the overall relative risk of developing CD in Caucasian populations was 2.4 (95% CI 2.0 to 2.9) for carriers of one mutant allele and 17.1 (95% CI 10.7 to 27.2) for two or more mutant copies. The risk varied with each mutation, Leu1007fsinsC generally carrying the highest risk and Arg702Trp the lowest. The overall proportion of CD cases attributed to the presence of mutant alleles in Caucasian populations was approximately 22% (50).

Further evaluation of *CARD15/NOD2* risk alleles have demonstrated a number of phenotypic associations. The major variants are associated with earlier age of onset (27,51,52) and ileal disease location (27-30,35,42,52-54). They are also suggested to be associated with fibrostenotic disease behaviour, (29,30,34,36,51,52,55) and negatively associated with pure colonic disease (27,30,35,42,43,51,52). In summary, the evidence to date suggests that the three risk alleles within the

**TABLE 1**  
Inflammatory bowel disease (IBD) susceptibility loci

Chromosome	HUGO ID	Phenotype	Associations
16q	<i>IBD1</i>	CD	<i>NOD2/CARD15</i>
12q	<i>IBD2</i>	IBD, especially UC	
6p	<i>IBD3</i>	IBD	HLA, TNF
14q	<i>IBD4</i>	CD	
5q	<i>IBD5</i>	IBD	<i>IBD5</i> haplotype, <i>OCTN1</i> and <i>OCTN2</i>
19p	<i>IBD6</i>	IBD	
1p	<i>IBD7</i>	IBD	
3p	<i>IBD9</i>	IBD	

CD Crohn's disease; HUGO ID Human genome organization identification; HLA Human leukocyte antigen; NOD2/CARD15 Nucleotide-binding oligomerization domain 2/Caspase activating recruitment domain 15; OCTN Organic cation transporter; TNF Tumour necrosis factor; UC Ulcerative colitis

*CARD15/NOD2* gene are correlated with CD which has an earlier onset, involves the ileum and is frequently fibrostenotic.

### OTHER IMPORTANT IBD LOCI

To date, *CARD15/NOD2* is the only confirmed IBD susceptibility gene identified. There are a number of other likely candidate genes and loci that have been described, although details remain less clear, and their roles less well characterized than *CARD15/NOD2*.

#### The *IBD5* locus on chromosome 5q31

The *IBD5* locus on chromosome 5q31 has received much attention recently. Aside from *CARD15/NOD2*, it is the next most relevant region for IBD susceptibility. The locus was initially identified as significant by two GWSs (56,57). Subsequent analyses refined the locus to a 250 kb risk haplotype (58). The association of this haplotype with IBD has been widely replicated in a number of independent populations (59-62). The *IBD5* risk haplotype has been principally associated with CD, although there have been some suggestions of a weak association with UC as well. Polymorphisms within the organic cation transporter (*OCTN1* and *OCTN2*) genes in the region have been recently put forward as the causative variant based on a combination of functional and genetic evidence (63). *OCTN1* and *OCTN2* are transporters that mediate transmembrane transport of carnitine and other organic cations (64,65). Although putative mechanisms have been suggested (65), no direct evidence is available to date to explain the role the variants of these genes could play in the pathobiology of IBD. Despite the efforts of several large centres, there has been an inability to replicate the findings that *OCTN1* and *OCTN2* polymorphisms are independently associated with CD, and these additional studies (66-69) support the possibility that the *OCTN1* and *OCTN2* SNPs are simply part of the extended haplotype in the region. This region is particularly difficult to fine map because it contains a significant degree of linkage disequilibrium, making it difficult to discern a causative allele from a marker allele coinherited with the disease-causing allele (58,70).

Phenotypically, the *IBD5* locus has been associated with earlier onset disease (56) as well as perianal disease (61,67). While most commonly reported with CD, an association with UC has been reported (62) with the strength of both associations possibly increased in the presence of a known *CARD15/NOD2*

variant (60,71) suggesting a positive epistatic interaction (the influence of one gene on the expression of another). With the uncertainty over the role of *OCTN1* and *OCTN2*, attention has focused on a number of additional genes located within the *IBD5* locus. These have included genes within the cytokine cluster, as well as interferon regulatory factor 1, *PDLIM* and proline 4-hydroxylase alpha polypeptide II, all of which are equally likely to contain the *IBD5* causal variant as the *OCTN* genes.

### THE MAJOR HISTOCOMPATIBILITY COMPLEX REGION (*IBD3*) ON CHROMOSOME 6P

Linkage of IBD (both UC and CD) to the *IBD3* region on chromosome 6 has been confirmed in a variety of GWSs (56,57,72-74). In a recent meta-analysis (75) of 10 such scans, it was the only locus that achieved genome-wide significance. The *IBD3* region contains the major histocompatibility complex genes (also referred to as the human leukocyte antigen [HLA]). The HLA complex is divided into three regions (class I, class II and class III) and contains a total of 224 densely packed highly polymorphic gene loci (76). Not surprisingly, the study of this area has proved very challenging, with various conflicting results. Several linkage and association studies (56,73,74,77-80) have implicated the HLA region in both IBD susceptibility and phenotype, although it has not been consistently confirmed in replication studies (81). A recent meta-analysis of 20 studies (82) highlighted both positive and negative associations with a variety of class II DRB1 alleles. For example, DRB1\*1502 is associated with UC across a variety of different populations. Despite extremely variable background prevalence (from less than 1% in Northern Europeans to approximately 25% in Japanese) the relative risk in each ethnic group is surprisingly similar (two to 4.5) (80,83-86). This pattern suggests the allele is a susceptibility variant.

However, genetic variation may not only contribute to susceptibility, but may also modify disease phenotype. Indeed, multiple-affected families show surprising concordance for disease phenotype, including age of onset, disease location, behaviour, need for surgery and extraintestinal manifestations (3,87-90). It is possible that HLA plays a greater role in determining final disease phenotype than initial disease susceptibility (76). For instance, DRB1\*07 is the most consistently replicated association with CD. It is specifically associated with ileal disease in the absence of a major *CARD15/NOD2* mutation (27,28,82,91). In comparison, DRB1\*0103 is a rare allele that has been associated with both UC and CD. In UC, it is the most consistently replicated HLA association. In UC, the association has been reported across a variety of ethnic groups in combination with various haplotypes, and is particularly strong in patients with extensive or severe disease (80,83,92-95). In CD, the allele is strongly associated with isolated colonic disease (27,28,91,96). Interestingly, this allele (along with HLA-B\*27) has also been associated with uveitis (97). In addition, DRB1\*0103 (as well as B\*27 and B\*35) has been associated with type I peripheral arthropathy (acute, self-limiting, pauciarticular large-joint arthritis associated with IBD relapses) while HLA-B\*44 has been associated with type II arthritis (symmetrical, seronegative, small-joint arthropathy unrelated to disease activity) (98).

Many of the genes in this region are also involved in the immune response and include the genes for TNF, lymphotoxin alpha and the heat shock proteins. Of these, TNF is certainly

the most intensely studied. Associations have been found with CD and promoter polymorphisms at positions -308, -857, -863 and -1031; however, their functional significance remains unclear (27,99-106).

### DROSOPHILA DISCS LARGE HOMOLOGUE 5 (*DLG5*) AND IBD SUSCEPTIBILITY

The pericentromeric region of chromosome 10 was identified as a potential IBD susceptibility locus in a European GWS. More recently, the region was refined to a haplotype block that contained only one gene: drosophila discs large homologue 5 (*DLG5*) (107). The data suggested an overall association for *DLG5* with IBD susceptibility rather than just CD, with a relative risk of approximately 1.5 (107). These findings have been, at least, partially replicated in some centres (108,109), but not in the majority of studies (67,110,111). *DLG5* is an attractive susceptibility candidate because it is putatively involved in maintaining epithelial integrity, and thus, its dysfunction would be consistent with an etiological model of impaired barrier function. However, much work remains to be performed to ascertain the role of this gene in IBD susceptibility.

### MULTIDRUG RESISTANCE-1 GENE AND IBD SUSCEPTIBILITY

The multidrug resistance-1 (*MDR1*) gene is a biologically plausible candidate gene for a number of reasons. First, *MDR1*-deficient mice are known to spontaneously develop enterocolitis when maintained in a specific pathogen-free environment (112). The gene's product, P-glycoprotein 170, is highly expressed in various epithelial surfaces including the intestine. Langmann et al (113) recently demonstrated a marked downregulation of this product in the colonic tissue of UC patients but not CD patients. Finally, the *MDR1* gene maps to chromosome 7q22 in a region that was previously identified by a GWS as being suggestive for linkage to IBD (114). To date, the most consistently reported association is with UC (115-118). Ho et al (115) described two haplotypes, one associated with disease susceptibility and the other disease protective. Current data suggest a specific association with extensive UC (115). The exact physiological role of this protein within the gut remains controversial. A variety of other case-control studies (119,120) have failed to detect any association between this gene variant and UC.

### CLINICAL IMPLICATIONS

The field of IBD genetics is in its infancy despite relatively rapid success in a number of areas. The integration of genetic testing into the clinic is still premature, however, it will not be long before genetic testing may become an important part of the initial workup of a patient with suspected or known IBD (Table 2).

The use of genetic testing to predict disease in presymptomatic patients is still not possible due to the relative lack of sensitivity and specificity of *CARD15/NOD2* testing. The same argument applies to genetic testing for diagnosis of IBD and the early presentation of IBD patients. It is more plausible that in the future, a panel of genetic and possibly serum markers will be tested to provide a measure of how likely a person is to develop IBD and what a patient's diagnosis is after symptoms have begun. Prognostic testing in individuals diagnosed with IBD is an area where molecular testing of genetic and serum markers

**TABLE 2**  
**Potential application of genetic testing in inflammatory bowel disease**

Prediction of disease susceptibility
Presymptomatic testing to detect 'at risk' individuals
Diagnosis
Prognosis
Choice of therapy and response to therapy
Disease prevention with specific interventions
Do not smoke?
Avoid unnecessary nonsteroidal anti-inflammatory drugs?
Probiotics administration?
Gene therapy?

may have the most potential current value. For example, it is known that *CARD15/NOD2*-positive individuals are more likely to have ileal disease and fibrostenotic disease, and are potentially more likely to proceed to an early ileal resection (121). If this is confirmed, then testing may allow the identification of those at risk of this complication and potentially target earlier or more advanced therapies to these individuals to prevent such complications. Again, a panel of genetic markers will likely be more useful for this indication. This type of predictive testing is evolving in serological marker testing where combinations of markers are associated with more aggressive small-bowel CD with a predilection to advanced disease behaviour (such as fibrostenotic or internal penetrating disease). Indeed, it is possible that the presence of such serum markers is genetically mediated and that ultimately a combination of genetic and serum marker testing will predict the course of the disease.

Pharmacogenetics is the study of how genetic variation influences an individuals' response to therapy (122,123). The hypothesis is that characterization of a specific genetic polymorphism will predict drug response and/or toxicity. It has been estimated that genetic variation can account for 20% to 90% of variability in drug disposition and effect (124). A classic example of the role of pharmacogenetics in IBD is the utility of thiopurine methyltransferase (TPMT) genotyping and the relationship to the metabolism of azathioprine/6-mercaptopurine. TPMT is one of the main degradation enzymes for these drugs,

and mutations within this gene have been associated with toxic side effects. However, the appropriate clinical application of this knowledge is still debated. While one might hypothesize that knowledge of the genotype could avoid toxicity, it is important to note that TPMT mutations appear to only account for approximately 10% to 27% of observed toxic reactions (125-128). Recent work with *MDR-1* introduced the potential ability to predict steroid resistance with the suggestion that there is an overexpression of *MDR-1* in the peripheral blood lymphocytes of steroid-resistant IBD patients (129). Hoffmeyer et al (130) have demonstrated an association with a specific variant of this gene (SNP C3235T) and its in vivo expression levels. In the future, it may be possible to predict a patient's steroid responsiveness from a genetic test (122,131). Conversely, as discussed earlier, an association was demonstrated with underexpression of *MDR-1* and the development of colitis. Pharmacogenetic studies are being undertaken in virtually all current IBD therapies, however, to date, there are minimal data available that would yield an impact upon clinical care.

### FUTURE DIRECTIONS

While each individual genetic risk factor identified thus far for IBD accounts for very little of the disease's overall heritability, the interaction between these and other genes as well as between genes and environmental risk factors is likely to play a very important role in disease pathogenesis and outcome. With advances in genetic technology leading to genome-wide association testing about to be completed in IBD, it is much more likely that all of the important genetic variants that contribute to IBD susceptibility will be identified. This will initiate an era of tremendous promise in the field of IBD research with the unique opportunity to make real advances in understanding the cause of IBD and to enable us to reach the goal of disease prevention and possibly a cure. While this is surely to take many years of painstaking work, the discoveries in IBD genetics will provide many ways to improve the lives of those living with IBD and make more tools available to those treating IBD. With improvements in diagnostic and prognostic testing as well as the development of novel drugs or drug tools to enable us to use existing therapies more effectively, the field of IBD genetics places us at the dawn of a new horizon in our efforts to better understand and manage IBD.

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