

## Advances in genetics of IBD: the NOD2/CARD15 gene

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### SUMMARY

During the last few years, significant advances have been achieved in the understanding of the pathogenesis of inflammatory bowel diseases (IBD). By gaining new insights, paradigms that seemed to be a safe basis of our knowledge on IBD genetics have recently become doubtful. This review discusses and summarizes recent developments in IBD genetics. Genetic factors play an important role in the pathogenesis of IBD, including both ulcerative colitis (UC) and Crohn's disease (CD). IBD, are characterized by an abnormal mucosal immune response to enteric flora in genetically susceptible individuals. It seems that they comprise complex genetic disorders with multiple contributing genes. Recent linkage studies have implicated several genomic regions as likely to containing IBD susceptibility genes, some observed uniquely in CD or UC, and others common to both disorders. The best replicated linkage region, IBD1 (16q12), contains the CD susceptibility gene, NOD2/CARD15.

**Key words:** IBD, NOD2/CARD15 gene, genetics, Crohn's disease, ulcerative colitis.

### INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are chronic, relapsing inflammatory diseases of the gastrointestinal tract, widely known as inflammatory bowel diseases (IBD). They are thought to result from an inappropriate activation of the mucosal immune system driven by intestinal bacterial flora.<sup>1</sup> Both UC and CD have a

worldwide distribution and are common causes of gastrointestinal morbidity in Western Europe and Northern America. Recent population based studies suggest that the combined prevalence of these diseases in Western countries approaches 400 per 100.000.<sup>2</sup>

Despite numerous studies performed over several decades, the cause of IBD remains poorly understood. Inflammatory bowel disease is thought to result from an inappropriate and ongoing activation of the mucosal immune system driven by the presence of normal luminal flora. This aberrant response is most likely facilitated by defects in both the barrier function of the intestinal epithelium and the mucosal immune system. Several clinical observations suggest that genetic factors contribute to susceptibility to inflammatory bowel diseases (table 1). Epidemiologic and linkage studies suggest that IBD are multifactorial, in which genetic and environmental factors appear to play an important role in disease development. In particular, the role for genetic factors was suggested by studies showing familial aggregation of IBD and greater concordance in monozygotic twins compared with dizygotic twins.<sup>3</sup> Over the past years, this evidence has been supplemented by molecular data from genome-wide linkage studies of multiply affected IBD families.

In 2001, three independent survey groups<sup>4,5,6</sup> reported the identification of the first CD susceptibility gene, named

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**Table 1.** Chromosomal regions in IBD genetic linkage studies

Disease	Linkage region	Putative association
IBD1	CD chr.16cen	NOD2/CARD15
IBD2	UC, CD chr.12q	None reported
IBD3	UC, CD chr.6p	MHC region, TNF
IBD4	CD chr.14q	None reported
IBD5	CD chr.5q	Cytokine 5q cluster
IBD6	CD chr.19p	None reported
IBD7	UC, CD chr.1p	None reported

NOD2 (nucleotide-binding oligomerization domain), re-named recently CARD15 (caspase activating recruitment domain), on chromosome 16q12. NOD2/CARD15 is located in the IBD 1 locus and is highly associated with CD, but not UC.<sup>4,5</sup> NOD2/CARD15 is expressed in peripheral blood monocytes<sup>7</sup> and dendritic cells and can be upregulated in intestinal epithelial cells by TNF.<sup>8</sup>

This article reviews the most recent developments in the genetics of IBD and explores how they might impact on clinical practice.

## **Genetic epidemiology of IBD**

### *Ethnic and racial studies*

Epidemiologic studies show that IBD incidence and prevalence vary significantly depending on geographic location and ethnic or racial background. In general, there is an increased risk for developing IBD in urban compared with rural areas, in cohorts with a higher socioeconomic class, as well as in developed rather than less developed countries. Many regions (Denmark, United Kingdom, Canada) show continued increase in CD incidence and prevalence, whereas rates for UC seem to be stable.<sup>9-12</sup> Incidence has been found to increase when populations emigrate from low-risk geographic areas to those with higher risk.<sup>13,14</sup>

Among Caucasians, the prevalence of CD and UC among Jews in the United States is significantly higher than in non-Jewish Caucasians.<sup>15</sup>

### *Family studies*

In population-based studies, approximately 5%-10% of all affected individuals with IBD report a positive family history (table 2), indicating that the greater risk factor for developing IBD is having other family members with the disease.<sup>16-18</sup>

### *Twin studies*

Monozygotic twin concordance for CD is reported as 42%-58%, whereas the dizygotic twin concordance is not significantly different from that for all siblings. The rates for UC range between 6%-17% for monozygotic

twins and between 0%-5% for dizygotic twins, respectively.<sup>19-21</sup>

The fact that disease concordance is significantly less than 100% among monozygotic twins indicates that there is a reduced penetrance for the IBD genotype, most likely owing to nongenetic factors, such as environmental triggers.

### *Genetic linkage studies in IBD*

Two broad, complementary approaches are typically utilized in genetic studies of IBD, namely genetic linkage and association studies.<sup>22</sup> Genetic linkage studies type families containing more than one affected member for the purposes of identifying general genomic regions shared in excess of statistical expectation. Genetic linkage typically implicates broad genomic regions, encompassing scores of potential associated genes. Once linkage is identified by genome-wide searches, the identification of specific disease gene(s) requires the use of genetic association studies.

Genetic association studies test for differences in allelic frequencies in patients compared to control individuals. Whereas genetic linkage studies typically implicate broad genomic regions containing scores of potential genes, in outbred populations disease associations are typically observed over much more limited regions containing only one to several genes. The extent and functional characterization of human genetic variation is the fundamental current challenge of the Human Genome project. If DNA specimens from separate, unrelated individuals are sequenced at a particular genomic location, the sequence would be largely identical. As a rough estimate, at approximately every 500-1000 base pairs a genetic variant, or polymorphism, would be observed between individuals. This most commonly involves a simple nucleotide substitution known as an SNP (single nucleotide polymorphism). Undoubtedly, the large majority of SNP have no functional consequences, but an important subset of them will result in altered function of the gene product which contributes to the continuum of normal phenotype variation.

**Table 2.** Genetic epidemiologic features of IBD

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- 5-10% of IBD patients positive family history
  - Relative risk to first-degree relatives of an IBD proband: approximately 15-fold
  - Monozygotic twin concordance is 42-58% (CD) and 6-17% (UC) compared to less than 5% (CD and UC) dizygotic twin concordance
  - 10-36% prevalence of IBD if both parents are affected
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A further subset of these functional SNP contribute to disease pathogenesis.<sup>23,24</sup>

Genetic linkage approaches were initially developed for monogenic disorders, where the extent of locus heterogeneity (i.e. different sets of genes contribute in different sets of patients) is relatively limited. The application of genetic linkage approaches for complex multi-genetic disorders such as IBD has been more challenging.<sup>25</sup> These chromosomal regions implicated in IBD genetic linkage studies are shown in Table 2 and in Figure 1.

### ***IBD susceptibility loci***

#### ***The IBD1 locus.***

The IBD1 locus, in the pericentromeric region of chromosome 16, represents the best replicated region, showing positive evidence for linkage only in CD and not in UC.<sup>2,26</sup>

#### ***The IBD2 locus.***

This locus on chromosome 12, represents a region where the linkage evidence may be relatively greater in UC compared with CD.<sup>2,26,27</sup>

#### ***The IBD3 locus.***

This locus on chromosome 6p encompasses the major histocompatibility complex and has been implicated consistently for both CD and UC in a number of linkage studies. Additionally, this region contains the TNF (tumor necro-

sis factor) gene, for which functional promoter polymorphisms affecting TNF expression have been reported.<sup>26</sup>

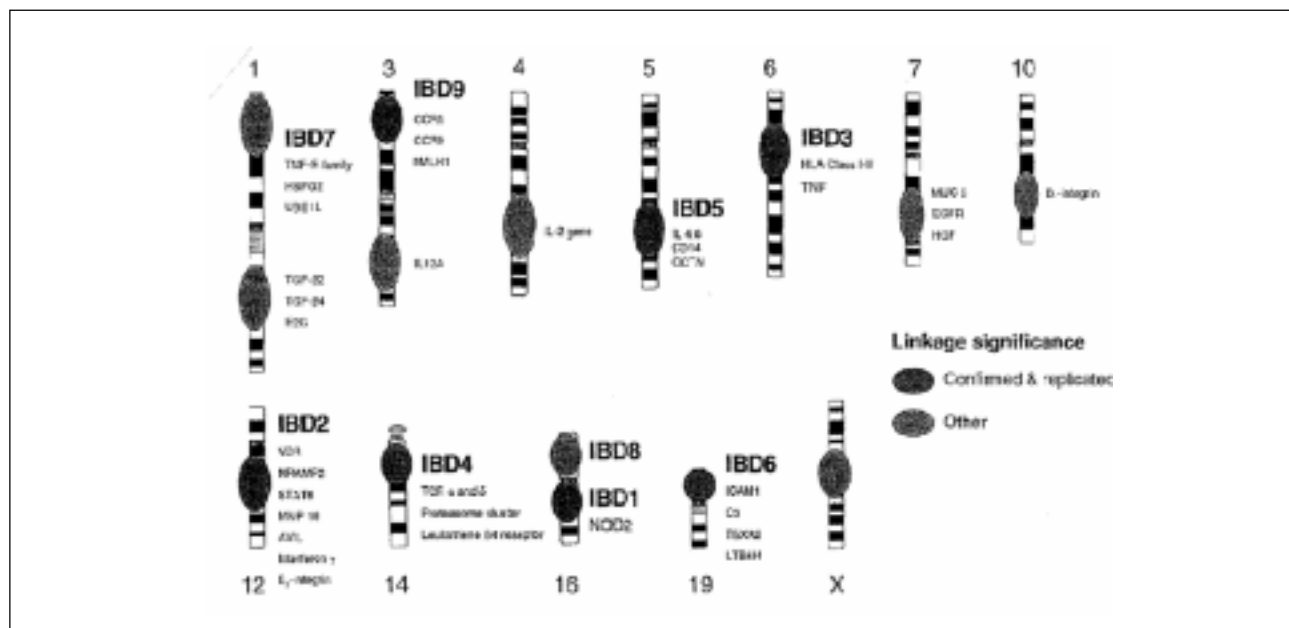
#### ***The IBD5 locus.***

This locus, at chromosome 5q31-q33 region, contributed to CD susceptibility in families with early-onset disease. This region contains a number of immunoregulatory cytokines that might be important candidate genes in the pathophysiology of CD: interleukins 3,4,5 and 14, as well as other candidate genes, such as colony-stimulating factor isoform 2 and the transcription factor, interferon regulatory factor, isoform 1.<sup>26</sup>

#### ***Other IBD loci.***

Significant linkage of IBD to chromosome 19p13 has been observed in Canadian sibling-pair families. Several candidate genes in this region are known, intercellular adhesion molecule 1, complement component 3, thromboxane A2 receptor and leukotriene hydroxylase. Furthermore, evidence for linkage to chromosome 1p was observed. Several genes located in this region are the TNF receptor family and caspase 9.

Finally, a suggestion of linkage to chromosomes 7q and 3p were observed, which are the sites of the mucin 3 gene (chromosome 7) and several genes on chromosome 3p for the receptors for hepatocyte growth factor and epidermal growth factor and an inhibitory guanine nucleotide-binding protein, GNAI2.<sup>26</sup>



**Figure 1.** IBD linkage areas, modified from Ahmad et al<sup>56,57</sup>

### The NOD2/CARD15 pathway

The well-replicated linkage of Crohn's disease to chromosome 16 stimulated efforts to identify the relevant susceptibility gene. The two main strategies employed, positional cloning and mutation screening of functional candidate genes in the region of linkage, both led to the identification of mutations in the NOD2 gene. Hugot et al.<sup>4</sup> detected a modest association of two alleles of a microsatellite polymorphism, D16S3136, with CD. They then sequenced the bacterial artificial chromosome containing D16S3136, and identified coding sequences of a gene which contained multiple SNPs, many of which were strongly associated with CD. This gene, originally named NOD2, was cloned independently by Ogura et al.<sup>28</sup> as a homologue of NOD1 and shown to be involved in the activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B). Since NOD2 mapped within the region of strongest linkage to IBD, this group screened it as a candidate for CD and detected association of a frameshift mutation 3020 insC (1007fs) with CD.<sup>5</sup>

The British/German group also screened NOD2 for mutations and detected the association of 1007fs with CD.<sup>29</sup> The presence of two caspase recruitment domains in NOD2 led to a change in nomenclature from NOD2 to CARD15.

NOD2/CARD15 is a member of the Apaf-1/Ced-4

superfamily of regulators of apoptosis. It is expressed intracellularly in monocytes (Figure 2) and macrophages where it appears to function as a sensor for bacterial products.<sup>30-32</sup> Three major NOD2 polymorphisms have been implicated in CD (Figure 3), denoted as single nucleotide polymorphisms 8, 12 and 13 (SNP 8, SNP 12, SNP 13). These correspond respectively to point mutations 2104C>T and 2722G>C and insertion 3020insC that results in premature stopping with a protein of 1007 instead of 1040 aminoacids. Of the three major variants, functional data are available primarily for the frame shift mutation,<sup>5</sup> where a diminished ability to activate NF- $\kappa$ B has been demonstrated. It is also possible that the other two variants may also alter the structure of the leucine-rich repeat domain at the C-terminal. These changes may result in abnormalities in bacterial recognition with implications for CD pathogenesis. These variants may also influence the familial versus sporadic occurrence of CD<sup>33,34-38</sup> disease location, appearance of stenoses and strictures and CD outcome.<sup>33,34-41</sup>

A recent meta analysis showed distinct risks for CD for the three common NOD2 variants, with narrow confidence intervals. Thus, SNP 8 increased the odds ratio by two-fold, SNP12 by three-fold and SNP 13 by four-fold in non-Jewish descent Caucasians. Furthermore, SNP 13 also has the strongest functional supporting data to date, exhibiting the most diminished ability to acti-

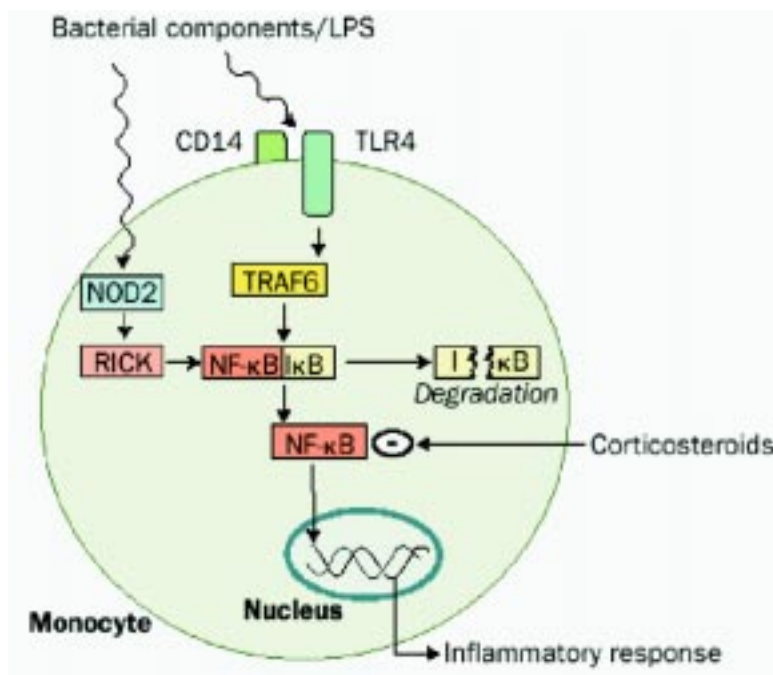
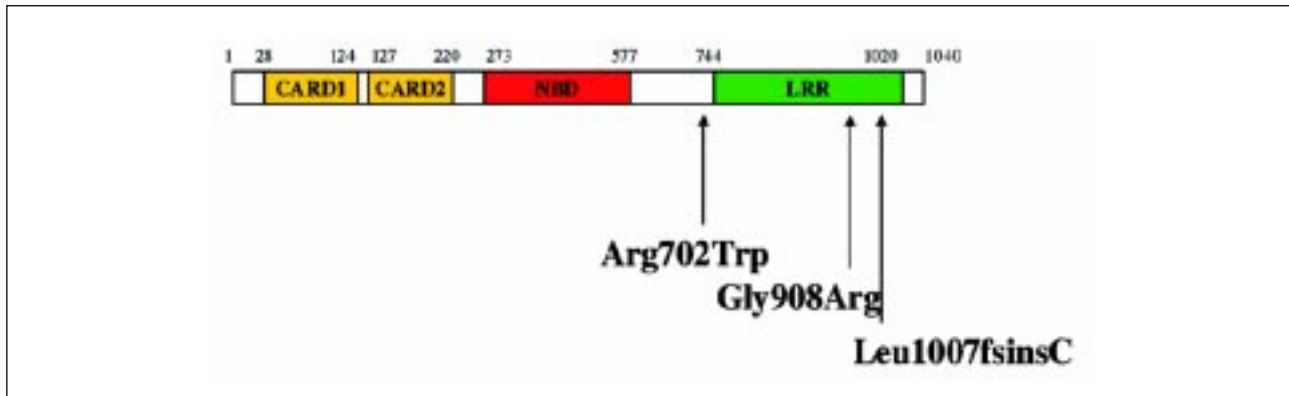


Figure 2. Monocyte innate immunity



**Figure 3.** Schematic representation of CARD 15 gene and the major mutations in CD

vate NF- $\kappa$ B compared to SNP 8 and SNP 12.<sup>42,43</sup> The corresponding estimates for Jewish descent patients followed the same risk order, but tended to be smaller.

The meta analysis also clarified the magnitude of the dose response relationship between the number of the high risk alleles and CD risk. Carriage of at least two variants increased the odds about 17 times in the studied populations. Nevertheless, these variants seem to confer considerable risk for CD. Most genetic risk factors for complex diseases have a much smaller impact.<sup>44-46</sup>

Furthermore, the high-risk alleles may influence primarily the disease location and then ileal disease increases the risk of stenosis. Allowing for these caveats, the observed strongest effect for ileal location, is consistent with the recently proposed involvement of ileal Paneth cells in the pathophysiology of NOD2 mediated disease susceptibility.<sup>47</sup> Paneth cells express NOD2 throughout the small intestine, with maximal expression in the terminal ileum, where Paneth cells abound<sup>47</sup> and the histopathological changes of CD are commonly encountered.

### ***Genes and environmental contributions to IBD***

#### ***Tobacco smoking***

Of the potentially relevant environmental factors implicated in IBD, the most established association is with smoking or tobacco exposure. Smoking has been shown to have a protective effect for UC, though having an adverse effect on the clinical course of CD.<sup>48</sup> Not only were active smokers less likely to develop UC than were nonexposed and nonsmokers, but the risk was also decreased in passive smokers.<sup>49</sup> This inverse association in UC and CD most likely is linked to differences in the pathogenesis of the two diseases.

The frequency of tobacco use appears to be similar between NOD2 carriers and noncarriers.<sup>50</sup> Multivariate analysis suggests that both tobacco use as well as NOD2 allele carriage independently increase the risk for developing ileal disease.

#### ***Mucosal immune system***

An interplay between genetic, bacterial and immunologic contributions in intestinal inflammation has been suggested by animal models of IBD.<sup>51</sup> The diversity of defects that predispose these animals to intestinal inflammation correlates with the importance of mucosal integrity in the maintenance of a healthy intestinal ecosystem, specifically, a network of interactions among the flora, the epithelial barrier and the immune response. Therefore, mutations in any one of a number of different genes in this network may disrupt the homeostasis of the mucosal immune system. Also, interindividual differences in epithelial barrier function, including the formation of an adequate mucus layer, may alter the subsequent exposure of intestinal microbes to the intestinal mucosal immune system.

There is considerable evidence, through animal models, that IBD is caused by genetically determined dysregulation of the mucosal immune response to luminal antigens derived from normal intestinal microflora.<sup>52</sup> Although the intestine is colonized normally by large numbers of bacteria, the intestinal mucosa is relatively free of adherent bacteria. However, a number of studies have established that patients with IBD have increased numbers of a variety of different bacterial antigens within the intestinal mucosa.<sup>53</sup> One study suggested that increases in the populations of specific bacteria such as *Escherichia coli*, *Enterococci*, *Bacteroides* and *Fusobacteria* may be important in postoperative recurrence of CD.<sup>54</sup> A sepa-

rate study showed that some mucosa-associated strains are capable of altering the permeability of cultured intestinal cell monolayers.<sup>55</sup> Therefore, genetic factors may contribute both to the increased penetration of bacteria and bacterial products, as well as an altered mucosal inflammatory response to those microbial products.

### Conclusions

The past decade has witnessed many advances in the understanding of IBD genetics. Recent epidemiologic data strongly suggest that IBD comprises a heterogeneous family of inflammatory disorders in which disease susceptibility, phenotype and response to therapy are determined by the complex interaction of genetic and environmental factors. However, it seems that the major contributing factor to IBD is genetic susceptibility. In fact, the greatest risk factor for disease is a positive family history. Through genetic linkage analysis and association studies, several candidate regions of genes have been identified for both UC and CD. Tremendous progress has been made in unraveling the contribution of genetic, microbial, cellular and molecular factors in the pathogenesis of IBD. Important advances toward understanding this process have been the identification of specific CD-associated NOD2/CARD15 genetic variants, as a major tool for understanding disease mechanisms, to develop molecular diagnosis and to better define personalized therapeutic options. The translation of this finding into clinical practice still remains a challenge.

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