

# Susceptibility Genes and Overall Pathogenesis of Inflammatory Bowel Disease: Where Do We Stand?

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## Key Words

Inflammatory bowel disease · Crohn's disease · Ulcerative colitis

## Abstract

The rapid accumulation of new knowledge on the genes, gene variations and genetic loci associated with both forms of inflammatory bowel disease (IBD), e.g. Crohn's disease (CD) and ulcerative colitis (UC), is shedding new light on the immunopathogenic mechanisms underlying these conditions. After the initial report of the association of *NOD2* mutations with ileal CD, a large number of additional genetic variants and loci has been found to be associated with both CD and UC, CD alone and, quite recently, UC-associated variants have also emerged. Much of this progress is due to the use of methods such as genome-wide associations (GWA) based on large numbers of reasonably well-characterized patient groups. Among several others, some of the most pathophysiologically relevant associations reported so far are with gene variants related to innate immunity, autophagy, apoptosis, Th1 and Th17 responses, T cell activation, and immunosuppression. Some of these associations have lent further support to previously construed disease mechanisms or disclosed brand new mechanisms, like in the case of the autophagy pathway. While this much progress is obviously welcome, it also brings new challenges. These include

the fact that all the gene mutations uncovered so far only account for a minority of all IBD cases, the variable distribution of gene mutations among worldwide IBD populations, and the still unknown effects of gene-gene and gene-environment interactions. Nevertheless, there is no question that genetic information will be quickly utilized not only for a better understanding of IBD pathogenesis, but it will also soon be incorporated into the armamentarium of better diagnostic and therapeutic tools.

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## Introduction

After half a century of continuous research the exact pathogenesis of inflammatory bowel disease (IBD) is still unclear, and one could rightfully ask the question of how long will it take to completely unravel its secrets [1]. One of the reasons explaining this disappointing state of affairs is that both forms of IBD, Crohn's disease (CD) and ulcerative colitis (UC), belong to the so-called group of 'complex diseases', indicating that numerous and diverse factors, conditions and mechanisms are reciprocally involved in highly complex biological networks underlying this class of disorders. Complex diseases affect a large portion of humanity and, although they are distinct and fall in many separate categories according to the organ or

system affected, they often share common predisposing factors and analogous mechanisms of tissue damage. Among the former are a huge number of genes and genetic mutations, which often determine not only whether the disease will appear or not, but also whether it will be mild or severe, of short or long duration, and how well the patient will respond to therapy [2]. More and more this seems to be true for IBD, which is characterized by a vast and heterogeneous constellation of manifestations and different outcomes. This review will briefly appraise the various components of IBD pathogenesis, and then will attempt to elucidate how the major genetic variations currently associated with CD and UC might contribute to the mechanisms of gut inflammation.

### Basic Components of IBD Pathogenesis

At present, the distinct components leading to CD and UC are fairly well defined, and there is general agreement that environmental, genetic, microbial and immune factors somehow interact closely with each other, the result of this interaction being a chronic inflammatory process that damages the gut and triggers symptoms [3–5]. The continuous increase in spreading of IBD worldwide leaves no doubts that environmental factors are at the root of the disease, but they alone cannot be blamed for directly causing gut inflammation [6]. Instead, it is far more likely that environmental factors, such as food, drugs, smoking, geography and social status, stress, and microbes in or outside the gut, act directly to skew the immune system towards pro-inflammatory responses, or do so indirectly by modulating genes that normally control the host's immune and intestinal homeostasis. The latter possibility appears increasingly viable and credible based on current developments and discoveries in the field in IBD genetics.

### Genetics of IBD

All diseases are 'genetic', but the degree of contribution by any given gene or gene combination is extremely variable, and the expression of any disease is also highly dependent on the balance of genetic versus environmental influences [7]. Some autoimmune/chronic inflammatory conditions, such as psoriasis, are under strong genetic influence, while others, like multiple sclerosis, are at the other end of the spectrum, with weak genetic but strong environmental pressure. IBD is probably in the

middle of the two extremes, with external and genetic factors both playing roughly equally important roles in disease pathogenesis.

Early investigation of IBD genetics focused on histocompatibility antigen (HLA) phenotypes, and reports of significantly increased or decreased HLA frequencies in UC and CD appeared in the literature [8]. About a decade later an association of HLA-DR2 with UC was reported in Japanese patients [9], followed by linkage analysis studies supporting an association between various HLAs and UC in European Caucasoid subjects [10]. At the same time, evidence of possible associations of immune genes with IBD started to emerge, such as an increased frequency of the allele 2 of the IL-1 receptor antagonist in patients with UC [11]. Although groundbreaking, these early studies were limited by the small number of subjects or families studied, a relative lack of patient homogeneity, and the intrinsic restrictions of a candidate gene approach. This situation has drastically changed with the realization that far larger and more homogenous populations need to be studied to obtain valid and interpretable results and with the advent of massive genome-wide association (GWA) screening approaches [12, 13]. When applied to IBD, these new methods wide opened the field of IBD genetics, and a large number of associations have so far been reported in CD and UC [14–18], as well as distinguishing genetic differences between these types of IBD [19, 20].

### The First Gene: *NOD2/CARD15*

In 1996, a GWA on two consecutive and independent panels of families affected by CD identified a putative CD susceptibility locus on chromosome 16 [21], and 5 years later specific variations of the *NOD2* gene on this chromosome were independently reported by two groups [22, 23]. These reports not only represented a milestone in IBD genetics, but also a lucky break. In fact, the product of the *NOD2* (also named *CARD15*) gene, which officially belongs to a family of genes regulating apoptosis, is an intracellular sensor of bacterial products, including those from the commensal enteric flora which, at the same time of the gene discovery, was increasingly being scrutinized as the possible target of the abnormal immune response occurring in IBD patients and animal models of IBD [24]. This connection triggered a frantic search aiming at understanding the function of the *NOD2*-encoded receptor and the underlying effects relevant to IBD pathogenesis. The specific bacterial moiety recognized by *NOD2* was

**Table 1.** Major IBD-associated genetic variations identified by genome-wide screens

Chromosome	Gene	Product function	CD	UC
1p31	<i>IL-23 receptor</i>	immune inflammatory response	+	+
5q33	<i>IL12b (p40)</i>	immune inflammatory response	+	+
9p24	<i>JAK2</i>	signaling	+	+
17q21	<i>STAT3</i>	transcription factor	+	+
18p11	<i>PTPN2</i>	T cell tyrosine phosphatase	+	-
9q32	<i>TNFS15</i>	immune inflammatory response	+	-
6q27	<i>CCR6</i>	chemokine receptor	+	-
3p21	<i>MST1</i>	macrophage chemotaxis	+	-
2q37	<i>ATG16L1</i>	autophagosome pathway	+	-
5q33	<i>IRGM</i>	autophagosome pathway	+	-
16q12	<i>NOD2/CARD15</i>	bacterial recognition	+	-
20q13	<i>TNFRSF6B</i>	inflammatory response, apoptosis	+	+
21q22	<i>PSMG1</i>	proteasome-related protein	+	+
12q12	<i>MUC19</i>	epithelial integrity	-	+
1q32	<i>IL-10</i>	immune inflammatory response	-	+

readily identified as muramyl dipeptide (MDP), a peptidoglycan component of the bacterial cell wall [25, 26]. Nevertheless, the consequences of recognizing MDP by products of a variant NOD2 receptor are still far from clear. In humans, CD-associated NOD2 variants have been reported to cause a decreased pro-inflammatory cytokine response to lipopolysaccharide (LPS) and peptidoglycan by monocytes and dendritic cells [27–29], beside being clinically associated with the ileal and fibrostenosing phenotype of CD [30]. This paradoxical response immediately raised the question of how a reduced production of pro-inflammatory mediators could cause gut inflammation. A preliminary hypothesis has been put forward that perhaps gut inflammation represents an overzealous adaptive immune response trying to compensate a defective antibacterial innate immune response, but this is far from proven yet. In animal models lacking or having a defective NOD2 gene, the situation is even less clear and a controversy is still raging on whether defective NOD2 function represents a loss or gain of function [31].

The existing confusion on the consequences and outcome of NOD2 defects in CD is actually very indicative of the difficulties awaiting IBD investigators aiming at understanding how any given gene defect leads to IBD. Considering the already substantial number of IBD genetic variants presently recorded, and the many more likely to be uncovered, this task appears overwhelming.

### Innate Immunity IBD Candidate Genes

The increasingly appreciated role of the intestinal flora as a target of abnormal immunity in IBD has led to an expanded investigation of its composition, the ways that enteric bacteria communicate with the gut, the physiological effects of this interaction, and the possible pathological consequences of when the crosstalk between gut microbiota and the host goes awry [32–35]. Microorganisms are recognized by pattern recognition receptors abundantly distributed on or inside cells of the innate immune system, particularly epithelial cells and cells of monocytic/macrophage lineage, which carry Toll-like receptors (TLR) and NOD-like receptors (NLR) [36]. In addition, a large number of other receptors, surface, signaling and secreted molecules also contribute to innate immunity [37, 38], with the ultimate goal of maintaining an effective but yet controlled immune response while avoiding inflammation [39]. Therefore, given its crucial role, it is not surprising that possible genetic defects in many innate immunity genes have been actively sought after, and several have been reported and claimed to be of pathogenic relevance (table 1) [16].

Evidence that innate immunity may be defective in IBD, particularly CD, has emerged [40], while potentially pathogenic bacteria continue to be proposed as specific etiological agents, like adhesive-invasive *E. coli* in ileal CD and *Mycobacterium paratuberculosis* [41, 42]. Because bacteria do utilize TLRs and NLRs to communicate with the host, it is reasonable to assume that genetic de-

fects of these receptors may lead to abnormal recognition of microbial antigens and secondarily inflammation. For instance, *M. paratuberculosis* is recognized by TLR2, TLR4 and NOD2 [43], and gene mutations in *TLR2* and *TLR4* have been found to be linked to increased susceptibility to this microbe in cattle [44], raising the theoretical possibility that humans with mutations in the same *TLR* genes may also be more prone to acquire or abnormally respond to *M. paratuberculosis* infection. Genetic variants of *TLR4* have also been detected in CD patients [45], providing additional basis for a defective function of this key innate immune pathway in this condition. Defensins, natural antimicrobial peptides produced by Paneth cells, are molecules also involved in innate immunity, and the hypothesis has been put forward that Crohn's disease is  $\alpha$ -defensin deficiency syndrome [46]. In fact, *NOD2* mutations are seemingly associated with diminished mucosal  $\alpha$ -defensin expression in CD [47], and in ileal CD there is a selective reduction of  $\alpha$ -defensin production only in those patients carrying the SNP10 mutation [48]. If so, mutations in the *HD-5*, *HD-6* and *HBD-2* genes on chromosome 8p23 could then be pathogenetically relevant. Along these lines, an extensive list of IBD candidate genes involved in innate immunity now waits to be investigated in greater detail (table 2).

### Autophagy Genes

Autophagy is a cellular 'cleanup' and nutrient stress (starvation) response system with a variety of homeostatic and disease-related effects. As a result of GWA, genetic variants in two autophagy genes, the *ATG16L1* and *IRGM* genes, have been recently identified and linked to CD [49–51]. Mutations of *ATG16L1* gene in humans with CD or in mice leads to fewer granules or diffuse granular contents in Paneth cells, while loss of autophagy in macrophages from *ATG16L1*-mutant mice results in aberrant IL-1 $\beta$  production [52]. Both findings potentially point to an altered interaction with the luminal flora and/or an exaggerated pro-inflammatory response. Other implications may also exist for defective autophagy in CD. *IRGM* induces autophagy to eliminate intracellular mycobacteria, and this could theoretically cause a putative *M. paratuberculosis* agent to persist in CD mucosa and cause inflammation [53]; moreover, autophagy in the thymic epithelium is essential to shape the T cell repertoire and establishment of tolerance, and defective autophagy early in life could lead to reactivity towards 'tissue-specific' self antigens [54]. Finally, autophagy and apoptosis are closely re-

**Table 2.** Innate immunity IBD candidate genes

Chromosome	Gene	Chromosome	Gene
7q22	<i>MUC3A</i>	11p15	<i>SIGIRR</i>
7q21	<i>MDR1</i>	16p13	<i>SOCS1</i>
3q13	<i>PXR/NR1I2</i>	11p15	<i>TOLLIP</i>
10q22	<i>DLG5</i>	16p13	<i>MEFV</i>
5q31	<i>OCTN1/2</i>	7p14	<i>NOD1/CARD4</i>
19p13	<i>Myosin IX B</i>	16q12	<i>NOD2/CARD15</i>
4q31	<i>TLR2</i>	19p13	<i>GRIM19</i>
4q35	<i>TLR3</i>	5q12	<i>Erbin</i>
9q33	<i>TLR4</i>	3p25	<i>TAK1/NR2C2</i>
1q42	<i>TLR5</i>	9q23	<i>HBD-5, -6</i>
4p14	<i>TLR6</i>	8q23	<i>HBD-2</i>
3p21	<i>TLR9</i>		

lated processes [55], and they may mutually inhibit each other and eventually result in defective apoptosis, an abnormality well documented in CD [56].

### Apoptosis-Related Genes

In addition to a potential defect in apoptosis related to autophagy, genetic variations have been reported in some genes that directly influence apoptosis. GWA associations have revealed polymorphisms in the *TNFSF15* and *TNFRSF6B* genes, which encode for a TNF-like factor (TL1A) and a TNF decoy receptor (DC3), respectively, the first having the ability to induce apoptosis and the second the ability to prevent apoptosis [57, 58]. Intriguingly, *TNFSF15/TL1A* is one of the ligands for *TNFRSF6B/DC3*, perhaps creating a dual defect in the regulation of apoptosis. It remains to be established whether these two specific genetic variations are actually related to the defective apoptosis of mucosal T cells in CD [59, 60], but they certainly create a reasonable basis for such scenario.

### Th1 and Th17 Response-Related Genes

Gene variants related to specific pathways of immune or inflammatory responses have also been reported in IBD. These genes include *IL-23R* and *IL12b*, which respectively encode for the IL-23 receptor (IL-23R) and the p40 (IL-12b) subunit belonging to the IL-12 family of cytokines [61, 62], and represent two of the most convincingly replicated gene associations in both CD and UC.

The biological relevance of these two gene products is very high because IL-23R and IL12b are essential for the development of T helper cells responses along the Th1 and Th17 pathways, whose end products are IFN- $\gamma$  and IL-17, respectively [63–65]. IFN- $\gamma$  and IL-17 are typically elevated in IBD, IFN- $\gamma$  more so in CD while IL-17 production is elevated in both CD and UC [66, 67]. At the moment it is not known how the multiple variants of *IL-23R* and *IL12b* may impact on the excessive Th1 and Th17 responses seen in IBD, and this is an area where intense investigation is currently under way [68]. Also of interest is the fact that *IL-23R* mutations are found in other autoimmune/chronic inflammatory conditions including ankylosing spondylitis, multiple sclerosis and autoimmune thyroid disease [69], suggesting that mutations in the IL-12 family of cytokine genes may be dominant in several immune-mediated disorders with a common epidemiological background and shared inflammatory pathways of tissue injury.

### T Cell Activation-Related Genes

Related to Th1 and Th17 responses are the JAK2 signaling molecule and the STAT3 transcription factor, which are involved in multiple activation pathways in a variety of cell types. Interestingly, variations in the *JAK2* and *STAT3* genes have been described and replicated in CD, UC, or both [70–72], another observation indicative of the possibility that major pathways of immune cell activation are genetically defective in IBD. Alternatively, a combination of genetic defects in cell activation, differentiation, regulation and effector function may be needed for full-blown clinical manifestations in IBD, or to determine the IBD subtype, the degree of disease severity, or response to therapy.

### Immunosuppression-Related Genes

On the opposite side of immune activation genes are those whose products are proteins that directly suppress immunity by deactivating stimulatory signaling pathways or indirectly through the secretion of soluble immunosuppressive molecules. Variants in this class of immunosuppressive genes have also been detected by GWA in IBD patients, including the *PTPN2* and the *IL-10* genes [62, 71, 73]. *PTPN2* is a tyrosine phosphatase expressed abundantly in T cells and its action is critical in counterbalancing the signals derived from the phos-

phorylation of several signaling molecules downstream of the T cell receptor activation pathway; IL-10 is a dominant immunosuppressive cytokine that counteracts the activation signals derived from a variety of immunostimulatory cytokines such as IL-2, IL-7, IL-15 and many others. Considering their foremost inhibitory function, it is easy to see how deficiencies in *PTPN2* or *IL-10* may lead to an overactive immune response and inflammation.

### Genetic Associations in CD and UC: Commonalities and Dissimilarities

An aspect that is very revealing of the burgeoning fascination with IBD genetics and the fast pace with which this type of research is taking place is the quickness with which CD and UC are becoming gradually separated at the genetic and genomic levels. Initially, most reports were focused on CD due to its generally stronger genetic overtone, as exemplified by the early detection of *NOD2* mutations. Then, as the use of GWA was expanded to both CD and UC, a greater number of loci was found to be associated with CD than UC, but recently a series of studies have appeared in the literature claiming the identification of genetic loci or variants specifically associated with UC. Susceptibility loci for UC have been recently identified at the *ECM1* locus and on chromosomes 1p36 and 12q15 [74, 75], as well as variants for the *IL-10* and *IL-10R* genes [73, 76]. The latter two are particularly interesting because their products are involved in mediation of immunosuppressive functions. Overall, it seems clear that the study of IBD genetics will in the near future define genes or groups of genes that are selectively associated with either CD or UC, or both [72].

### Limitations and Challenges of Current IBD Genetic Studies

While precipitous progress is occurring in the field of IBD genetics, the rapid accumulation of abundant but purely observational data is not accompanied by an equal rapid progress in understanding the biology of the newly discovered genes, variants and loci.

While it is possible to speculate and start investigating the prospective implications of the several gene variants linked to IBD, the sheer number of these associations is in itself an enormous obstacle to surmount. More than 30 distinct susceptibility loci have been defined for CD alone

**Table 3.** IBD candidate genes of uncertain significance

Chromosome	Gene	Name, function
5p13	<i>PTGER4</i>	prostaglandin E receptor 4 (G-protein-coupled receptor family member)
10q21	<i>ZNF365</i>	Zinc finger protein 365
10q24	<i>NKX2-3</i>	NK2 transcription factor related (NKX transcription family member)
1q22	<i>ITLN1</i>	intelectin 1 (galactofuranose binding)
6p22	<i>CDKAL1</i>	CDK5 regulatory subunit-associated protein 1-like 1
12q12	<i>LRRK2</i>	leucine-rich repeat kinase 2 (leucine-rich repeat kinase family member)
17q12	<i>ORMDL5</i>	ORM1-like 3 ( <i>S. cerevisiae</i> )
2p23	<i>GCK3</i>	glucokinase (hexokinase 4) regulator
2p16	<i>PUS10</i>	pseudouridylate synthase 10
6p25	<i>LYRM4</i>	LYR motif containing 4
6p25	<i>SLC22A23</i>	solute carrier family 22, member 23 (transmembrane uniporter, symporter, antiporter family member for organic ions)
Plasmid Ip28-1	<i>BafACA1_F30</i>	(no official name/ <i>Borrelia afzelii</i> ACA-1 strain)

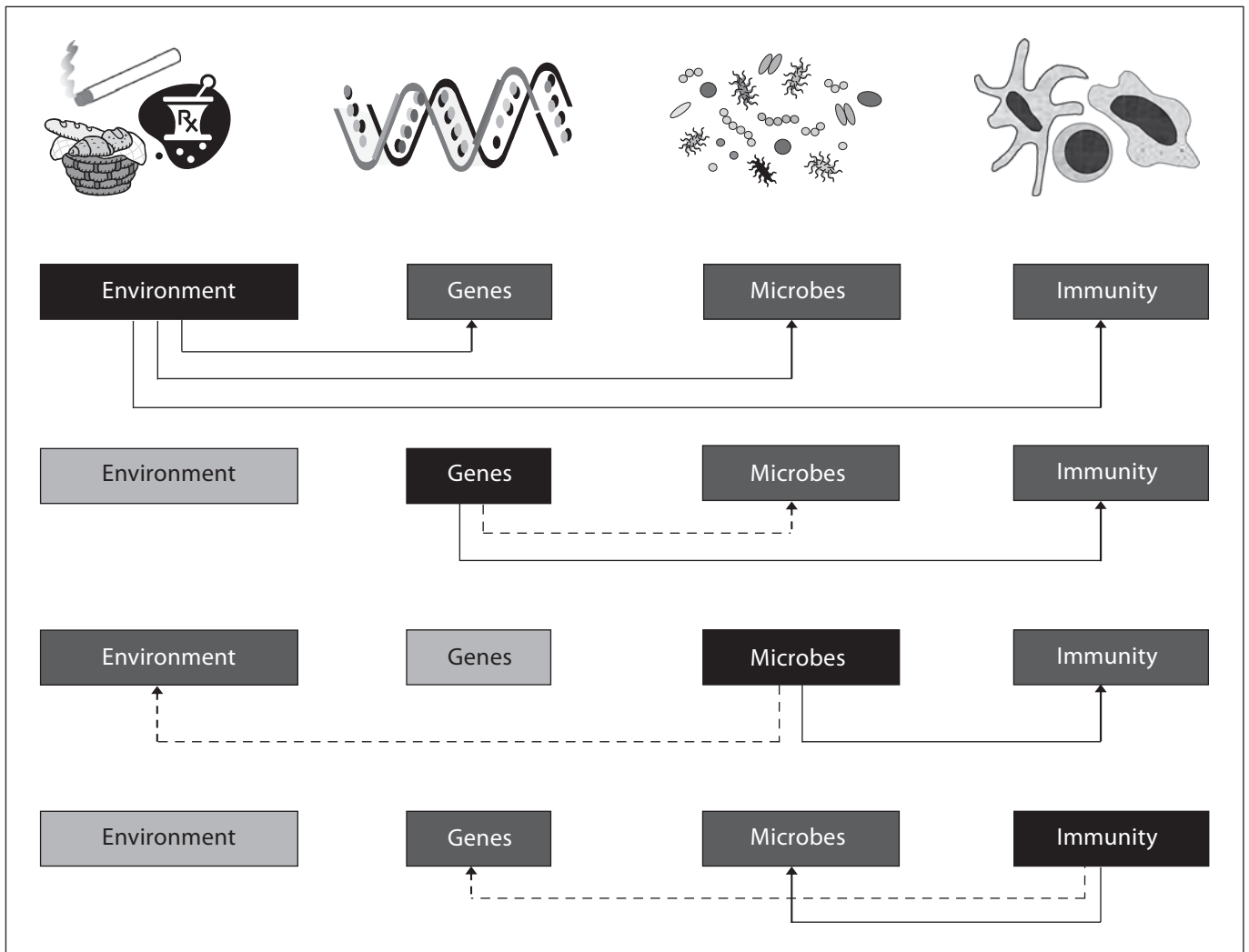
[70], and at present around 40 IBD-associated genes and loci have been described. Although many of them, at least conceptually, make some sense based on current knowledge of IBD pathogenesis, it will take considerable time and resources to work out how each variation may impact on triggering or maintaining the disease. Additionally, there is a substantial number of other IBD candidate genes detected by GWA that do not readily make biological sense, and several are just listed in various gene databases without any information on their possible biological function (table 3).

Another important issue is the distribution and frequency of IBD genes in the population at large. While *NOD2/CARD15* mutations appear to be equally distributed among patients with white, black and Hispanic background [77], the same mutations are not associated with CD patients in Japan or China [78, 79]. These observations are important because they indicate that other genes may predispose to IBD in various populations worldwide, and the mechanisms underlying gut inflammation may be distinct from one ethnic group to another.

One more challenge in interpreting the multiple IBD gene association comes from the complex interactions that normally occur among genes, very much like those that occur among cells, cytokines and signaling molecules. The phenomenon of gene-gene interaction, also called epistasis, can be investigated by using a variety of mathematical and statistical models, but these in turn have their own drawbacks: first, statistical analyses test hypotheses regarding quantities, not biological respons-

es; second, statistical interactions do not imply biological interactions; third, the interactions of 2 (or more) genes cannot be inferred from the individual action of each gene [80]. For instance, one is spontaneously compelled to assume that the risk of IBD is further increased when a patient harbors two unrelated IBD genetic variations. This instinctive but naive assumption is based on the independent action of each gene per se, but one cannot necessarily expect the same effect when the two genes interact in vivo in the presence of other modifying genes and innumerable other factors derived from both the endogenous and exogenous environment [81].

Thus, at least for the time being, an answer to the investigation of the biological significance of the numerous IBD genetic variations must rely on the systematic investigation of each variation by traditional in vitro testing of relevant cells derived from patients carrying the mutation of interest, complemented by in vivo studies with animals deficient in the specific gene of interest (knock out), animals overexpressing the gene (transgenic), and animals where the human variant has been introduced (knock in). Considering the already sizeable number of genes of potential interest to IBD, the feasibility of such demanding approach becomes questionable. The Human Genome Project, which looks into global responses, may alleviate some the investigational burden, but we also have to learn how to discover and integrate global networks among the environmental, genetic, microbial and immune components of IBD pathogenesis, each one of them influencing the action of the others (fig. 1).



**Fig. 1.** Global networks among components of IBD pathogenesis.

### Conclusions and Therapeutic Implications

Taking into consideration the large amount of new data on IBD genetics and the massive pre-existing information on IBD pathogenesis, both of which are constantly growing, how can we manage the IBD information overload? Obviously, novel and more effective approaches and systems are needed. Genomic analyses of differentially expressed genes in normal, CD and UC intestine will help [82, 83], particularly when integrated with the results of GWA studies. This combination of molecular classification and gene expression prognostic signatures may predict outcome and pave the way to improved therapeutic strategies, as it is currently postulated for breast

cancer [84]. Selection of therapeutic targets must also be improved by creating detailed genomic profiles in well-defined experimental systems to allow the identification of dominant genes and their regulatory elements by bioinformatics analysis, such as genes encoding central transcription factors. Pharmacogenomic studies must also be actively implemented in IBD, so that accurate drug targets, anticipated side effects, and optimal response to medications can be reliably predicted in selected groups of patients carrying precise IBD genetic patterns [85]. As the future unfolds, new solutions will certainly emerge.

The integration of knowledge derived from genetic and genomic analyses still seems out of reach to the clinician and patient alike, but in reality the incorporation of

this new knowledge into clinical practice is much closer than it appears to be. If one considers that only a decade ago the intended use of biologicals was still completely novel and under a cloud of ignorance and suspicion, its current routine use and the desire for newer, better and more powerful biologicals is nothing short of remarkable, and represents a strong testimonial of how quickly innovation coming from the bench gets absorbed into day-to-day clinical practice. The very same phenomenon will inevitably happen with information coming from genetic and genomic analyses, which is already transforming our current thinking of IBD pathogenesis. At the end of the 19th century Sir William Osler stated that ‘if were not for

the great variability among individuals, medicine might as well be a science and not an art’. Today, at the beginning of the 21st century, it is perhaps time to modify this statement and propose that ‘if were not for the great variability among genes, medicine might as well be an art and not a science’.

### Disclosure Statement

The author declares that no financial or other conflict of interest exists in relation to the content of the article.

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