Genetic susceptibility for Inflammatory bowel diseases
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Chapter 2

Inflammatory bowel diseases and genetics: current affairs

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Abstract

Tremendous progress has been achieved in unraveling the genetic etiology of inflammatory bowel diseases (IBD) comprising ulcerative colitis (UC) and Crohn’s disease (CD). It has led to the discovery of mutations in NOD2/CARD15 associated with ileal CD. It is only partially understood how mutations in NOD2/CARD15 lead to CD. Mouse models, in vitro data and studies in humans offer conflicting data whether there is a loss or gain of function of NOD2 in CD. Through the conductance of genome wide scans and hypothesis driven candidate gene studies several additional genes have been identified. Only few of these genes are currently being recognized as potential disease causing or disease modifying genes. Promising candidate genes include Toll like receptor 4 (TLR4), Multi Drug Resistance 1 (MDR1), NOD1 (CARD4), HLA DRB1*103, DLG5 as well as the IBD5 locus including members of the organic cation transporter cluster 1 and 2. For future genetic research accurate phenotyping of patients is very important and large population based cohorts are needed. Although genetic research has not yet led to better prediction of the disease course, development of malignancy or patient selection for medical therapy, remarkable progress has been made in the understanding of the pathogenesis of IBD. Eventually, genetic research may be able to classify different disease phenotypes on a more detailed molecular basis and may provide important contributions in the development of new therapeutic approaches.
Introduction

In the past decade, tremendous progress has been achieved in unraveling the genetic etiology of inflammatory bowel diseases (IBD) comprising ulcerative colitis (UC) and Crohn’s disease (CD). By conducting genome linkage studies, several susceptibility loci for IBD have been identified termed IBD1 to IBD7 (figure 1). Through fine mapping of these susceptibility loci as well as by hypothesis driven candidate gene studies, the CARD15 gene encoding for the NOD2 protein on IBD1 has been found to be strongly related to CD susceptibility. Since IBD is considered a multigenic disorder, many other candidate genes have been studied in addition to CARD15. None of these genes has been as consistently replicated as CARD15 and probably other disease susceptibility genes will not be discovered as straightforward as CARD15, due to low penetrance and complex gene-gene and gene-environment interaction. Promising candidate genes include Toll like receptor 4 (TLR4), Multi Drug Resistance 1 (MDR1), NOD1 (CARD4), HLA DRB1*103, DLG5 as well as the IBD5 locus including members of the organic cation transporter cluster 1 and 2.

Figure 1
Inflammatory Bowel Diseases (IBD) associated genes and susceptibility loci for IBD on the human genome, termed IBD1 to IBD7.
In addition to the search for new IBD susceptibility genes, one of the main research themes in IBD focuses on the function of NOD2 in CD. Although many questions have been answered, it still remains unknown whether there is loss or gain of protein function in patients carrying mutated NOD2. We will summarize current topics in IBD genetics, focusing on NOD2 functionality and novel candidate genes.

Innate immunity

Since the discovery of NOD2 in CD susceptibility, the innate immune system has been studied extensively. Current research, regarding the innate immune system in IBD, is focusing on NOD2 functionality and new candidate genes for IBD susceptibility.

Function of NOD2

NOD2 is composed of two N-terminal caspase recruitment domains (CARDs), a nucleotide binding and oligomerisation domain and ten C-terminal leucine rich repeats (LRRs) (figure 2). It is mainly expressed in macrophages, neutrophils and dendritic cells as well as in intraepithelial Paneth cells that are located in the crypts of Lieberkühn in the ileum. NOD2 is part of the innate immune system and is a pathogen associated molecular pattern (PAMP) receptor that recognizes muramyl dipeptide (MDP) which is a part of bacterial peptidoglycans. The three variants associated with IBD are all located in the LRR region which is the binding site for MDP. They consist of two missense mutations (R702W and G908R) and one frameshift insertion mutation (L1007fsinsC). The L1007fsinsC mutation causes a truncated protein, suggesting that a defect in bacterial recognition might be involved in CD. Interestingly, a mutation in NOD2 located in the central nucleotide binding domain instead of the LRR region is associated with Blau’s syndrome, a rare autosomal dominant disorder with granulomatous arthritis, uveitis and skin rash, implying different pathological pathways. CD is characterized by an increased activity of NF-κB and NOD2 has been shown to have a role in the activation of NF-κB. However, the precise mechanism, how mutations in NOD2 and subsequently activation of NF-κB leads to susceptibility of IBD is still only partially understood. Studies in NOD2 deficient mice have given contradictory results and one seemingly simple question whether there is loss or gain of function in CD associated NOD2 variants remains to be answered. In a recent study with insertion of mutated NOD2 alleles in mice, Maeda et al. showed that mutant mice had increased activation of the NF-κB pathway, increased secretion of IL-1β and were more susceptible to bacteria-mediated experimental colitis, suggesting a gain of function. On the other hand, a study by Watanabe and co-workers showed that NOD2 functions as a negative regulator of TLR2-mediated T helper type 1 response. NOD2 deficient mice showed increased TLR2-driven activation of NF-κB, particularly of the NF-κB subunit c-Re1, whereas intact NOD2 signaling inhibited TLR2-driven activation of NF-κB. In an additional study, NOD2 deficient mice had decreased expression in intestinal Paneth cells of cryptdins, which are mouse homologues of human α-defensins. These α-defensins are important mem-
bers of the innate immune system. NOD2 deficient mice were susceptible to bacterial infection via the oral route but not through intravenous or intraperitoneal exposure, suggesting an important role for NOD2 as a regulator of bacterial immunity in the intestine. Indeed, reduced expression of α-defensins is observed in Paneth cells in subjects with CD, which is even more pronounced in patients with NOD2 mutations. Loss of function of NOD2 may lead to enhanced mucosal invasion of bacteria resulting in an increased inflammatory response. Still, mouse models, in vitro data and studies in humans offer conflicting data and many additional studies are currently being undertaken to give an answer to the question how mutations in the LRR region of NOD2 lead to CD.

**NOD1 and Toll Like Receptors**

Since the identification of NOD2 in CD susceptibility, many further candidate gene studies were performed in genes involved in the innate immunity. The most promising results, although data have been conflicting and not solidly reproduced, suggest an association of NOD1/CARD4 and TLR4 with IBD. NOD1 is expressed in the epithelium of the small and large intestine and is fairly similar to NOD2, but differs by the presence of only one CARD. Its LRR functions as a pattern recognition receptor for diaminopimelic acid present in gram-negative bacterial peptidoglycans. Activation of NOD1 activates NF-κB and enhances apoptosis.

In addition, the gene encoding for NOD1 (CARD4) is located on chromosome 7p14, previously recognized as an IBD susceptibility locus. Though a previous study did not show an association with IBD, NOD1 has recently been implied in IBD susceptibility. McGovern et al. found that the deletion allele of a complex functional insertion-deletion polymorphism was associated with early age of onset IBD and extraintestinal manifestations. The same study showed that haplotypes in the terminal exons of CARD4 were also significantly associated with IBD. Again, these data need further confirmation.

The Toll Like Receptors are important members of the innate immunity. Different TLRs recognize selectively PAMPs of different classes of microorganisms. TLR4 recognizes lipopolysac-
charidases which are components of the cellular wall of gram negative bacteria. TLR4 is upregulated during intestinal inflammation in macrophages, dendritic cells and epithelial cells in IBD. Upon binding with LPS it forms a complex with CD14 at the surface of monocytes and neutrophils leading to NF-κB activation and the release of inflammatory cytokines. The Asp299Gly mutation is located in the extracellular LRR domain of TLR4 and is associated with decreased responsiveness to endotoxins in humans. This mutation has been found to be associated with CD in two studies and with IBD in one Belgian study. A second mutation Thr399Ile which is in strong LD with Asp299Gly has also been implied with IBD susceptibility. These results have not been confirmed in several other studies. In a study by our own group, association with the TLR4 region was found by haplotype analysis of microsatellite markers surrounding the gene but not with Thr399Ile or Asp299Gly. This finding implies that the Thr399Ile and Asp299Gly polymorphisms could be merely in LD with other disease associated variants in the TLR4 region, instead of being causative themselves.

A recent study found no genetic association of TLR1, TLR2 and TLR6 with disease susceptibility but an association between variants of these TLRs and a specific phenotype of extensive colonic disease in CD or UC was established.

The HLA region

The HLA region on the short arm of chromosome 6p (IBD3) was identified as a disease susceptibility locus in several genome wide scans as well as in a recent meta-analysis of 10 genome wide scans. This area harbors a total of 224 highly polymorphic genes, many of which appear to have immunoregulatory functions. HLA proteins present peptides to T-Cell receptors and are divided in class I and II HLA proteins. The HLA class II proteins play a central role in the immune response, are expressed on specialized immune cells and consist of an α-chain and a β-chain that form a groove by which the antigen peptide is presented to the T cell receptor. The three genes encoding for these α- and β-chains are HLA-DP, HLA-DQ and HLA-DR which are highly polymorphic. HLA class II genes have been extensively studied in association studies for IBD. A meta-analysis of data published until 1998 showed a positive association with UC of HLA DR2, DR9 and DRB*103 and a protective association with DR4. For CD, HLA-DR7, DRB3*0301 and DQ4 showed a positive association whereas HLA DR2 and DR4 showed a protective association. The association with HLA-DRB*103 has been confirmed in European cohorts and seems to be associated with extensive disease in UC and in CD with pure colonic involvement. HLA-DRB*103 seems to be a potentially important contributor to colonic involvement of IBD. However, the rare variant of HLA-DRB*103 has a very low frequency in the general population thereby minimizing its possible clinical value. Polymorphisms for HLA-DRB1*1502 which are found in populations with variable ethnic background have been associated with UC. HLA DRB*07 has been consistently replicated in CD patients with ileal localisation. Interestingly, patients carrying the risk associated allele for HLA DRB*07 did not carry any of the three NOD2 mutations, stressing the fact that there is a great genetic heterogeneity in CD,
even within specific phenotypes such as ileal localisation. Tumour Necrosis Factor-α (TNFα) is a proinflammatory cytokine that plays an important role in the inflammatory processes involved in IBD. The importance of TNFα is highlighted by clinical efficacy of treatment with anti-TNFα monoclonal antibodies in CD. TNF polymorphisms have been extensively studied in the HLA class III region. Several polymorphisms in the promoter region are known, but functional data are lacking and genetic association studies have been very inconsistent. Another interesting finding, although not in the HLA region, is the recently found association with the Tumour Necrosis Factor superfamily member 15 (TNFSF15) on chromosome 9q33 in a Japanese and a UK cohort. The function of TNFSF15 remains to be elucidated but it is involved in NF-κB activation and induction of apoptosis.

In conclusion, several genes in the HLA complex are involved in IBD susceptibility, but results of genetic research have been conflicting because of the high density of genes in this region, a high degree of LD resulting in highly conserved haplotypes and complex gene-gene interactions with genes outside the HLA region.

**Novel candidate genes**

*IBD5*

A genome wide screen in affected Canadian families identified linkage with a region at chromosome 5q31-q33 (IBD5) with a length of approximately 250 kb conferring susceptibility for CD with early age of onset. This region contains a number of potentially interesting genes, including several genes encoding for immunoregulatory cytokines. However, identifying the causative gene(s) in the IBD5 locus has been hampered by the high extent of LD in the region. Recently, two functionally relevant mutations in the carnitine / organic cation transporter (OCTN) genes on the IBD5 locus were shown to be associated with CD. The T substitution of C1672 in exon 9 of the SLC22A4 gene encoding for OCTN1 and a G to C substitution at position -207 in the promoter region of SLC22A5 gene encoding for OCTN2, were identified. Together, these polymorphisms form a 2 allele risk haplotype associated with CD susceptibility. Gene-gene interaction with mutations in the CARD15 gene is suggested. These results have been replicated in a German population even though the association was much weaker than in the original study of Peltekova et al. This is in concordance with the findings that the IBD5 locus as a whole is less important in European populations compared to the original Canadian cohort. Other studies did not find an association between OCTN polymorphisms and CD, though in one study an increased risk in a subgroup with perianal Crohn’s disease has been found. Relatively little attention has been given to these genes before, due to the lack of a sensible explanation for a role in the pathogenesis of IBD. In the paper by Peltekova et al. preliminary functional experiments were performed demonstrating that the two single nucleotide polymorphisms (SNPs) impair function of the OCTNs, resulting in reduced carnitine transport, but convincing functional data remain scarce.
In addition to the lack of solid replication studies and functional data, it is also difficult to understand how two mutations in two adjacent genes produce IBD susceptibility. It still remains to be seen whether the \textit{SLC22A4} and \textit{SLC22A5} genes encoding for OCTN1 and OCTN2 are the causative genes for IBD susceptibility at the IBD5 locus or that they are merely haplotype tagging SNPs in strong linkage disequilibrium with other disease associated genes. Larger population based cohorts and functional data are needed to answer this important question.

\textit{DLG5}

Recently the \textit{DLG5} gene (Drosophila Discs Large Homologue 5) has been recognized as a novel susceptibility gene for CD and IBD.\textsuperscript{19} \textit{DLG5} is important in maintaining the epithelial structure and genetic variants could result in impaired intestinal permeability.\textsuperscript{73} Stoll \textit{et al} identified \textit{DLG5} by refining their previously defined linkage region on chromosome 10q23. It is worth mentioning that this region has not been confirmed in other genome wide scans. Genetic variants in \textit{DLG5} were identified by using a positional cloning strategy. Two haplotypes were involved in IBD and CD susceptibility. A SNP 113 G→A, resulting in an aminoacid substitution R30Q, was positively associated with IBD and CD patients in a case control study. A second haplotype, identified by several other tagging SNPs was protective for IBD. Gene-gene interaction with \textit{CARD15} in CD was detected by a significant difference in association of the 113A variant in patients carrying the risk alleles for \textit{CARD15} compared to patients not carrying these alleles. Only one published study has been able to reproduce these results.\textsuperscript{74} Initial enthusiasm has been discouraged by several studies from Europe and Japan, failing to show any association for \textit{DLG5} with IBD.\textsuperscript{75-78} In the same way as for OCTN, large population based case-control studies are needed to elucidate the role of \textit{DLG5} in IBD susceptibility.

\textit{MDR1}

P- glycoprotein-170 (Pgp170) is encoded by the multi drug resistance gene (\textit{MDR1}). It was initially recognized to be responsible for resistance to cytotoxic drugs in cancer cells. It is an ATP binding cassette (ABC) transporter which is highly expressed in the intestinal epithelium. There is mounting evidence that Pgp170 is an important factor in host-bacterial interactions and in maintaining intestinal homeostasis.\textsuperscript{79} Mice lacking the \textit{MDR1} gene spontaneously develop colitis which is histologically similar to UC.\textsuperscript{80} In combination with the fact that \textit{MDR1} is located on chromosome 7q22 which was identified as an IBD susceptibility locus in one UK cohort \textit{MDR1} appears to be a good candidate gene.\textsuperscript{10}

Two exonic SNPs C3435Tand G2677T/A are involved in altered activity and expression of Pgp170. Schwab \textit{et al}. found the T allele and TT genotype of the C3453T SNP to be associated with UC and not with CD.\textsuperscript{16} This association with UC has been confirmed in a Slovenian and Scottish cohort and with IBD in a mixed North American population.\textsuperscript{81-83} This is in contrast with two other studies that could not replicate these positive findings.\textsuperscript{84 85} In a large case-control study of Dutch patients in our own center, we did not find an association with the \textit{MDR1} gene and IBD, UC or CD, neither on a single locus and haplotype association analysis nor with the haplotype sharing statistics.\textsuperscript{86}

Although the \textit{MDR1} gene holds promise as being involved in IBD susceptibility, there is doubt whether the previously found associations are true positive results.
Conclusions and future perspectives

Research in the genetic background of IBD is a rapidly evolving field and has helped unravelling pathophysiological processes involved in complex multifactorial diseases as IBD. Important progress and insight in the pathogenesis of IBD has been made by the discovery of NOD2/CARD15. In fact, the discovery of the CARD15 gene in IBD has been one of the success stories in genetic research in complex genetic diseases. The exact mechanism how NOD2 is involved in IBD susceptibility is unknown to date, but genetic research has helped us to focus research at signaling of bacterial products in both epithelial and immune cells in the gut.

Furthermore, numerous candidate gene studies have been performed which has resulted in several positive and negative associations for IBD. Many of these studies are hampered by small sample sizes and hence the lack of power to detect low penetrant genes or complex gene-gene interactions. Only few of the identified genes are currently being recognized as potential disease causing or disease modifying genes. To establish true positive associations, large population based case-control studies and functional studies are needed in the future. It is therefore encouraging that collaboration between centers and countries is initiated in IBD genetics.

In addition to susceptibility for IBD, genetic variations may account for disease expression including disease location, clinical behaviour and response to therapy. For that reason, one important “current affair” in IBD genetics is the reliable phenotyping of patients. Since the successful connection of NOD2 with ileal CD and the increasing number of serological markers associated with different subsets of IBD, it is recognized that different phenotypes of CD or UC patients are characterized by different molecular and serological markers. For future research, it is therefore of utmost importance that patients are accurately phenotyped according to a well defined clinical classification scheme. The Vienna classification is frequently used for genetic studies in CD and includes age of onset (A), disease localisation (L) and diseases behaviour (B). A number of studies have validated this classification; however, several considerations have led to an update of the Vienna classification system during an expert meeting in Montreal in 2005. The main modifications were the introduction of an early age of onset category (< 16 years), the possibility of co-classification of upper gastrointestinal involvement and the inclusion of perianal disease as a disease modifier instead of being a form of penetrating disease. The latter is an important modification because it is recognized that there is no clear association between perianal disease and intra-abdominal penetrating disease. The aim of rigorous phenotyping is to include serological and genetic markers into the clinical classification system to stratify patients and eventually to predict disease course and response to medical therapy.

Although genetic research has not yet led to better prediction of the disease course, development of malignancy or patient selection for medical therapy, remarkable progress has been made in the understanding of the pathogenesis of IBD. Genetic research holds a strong promise for the future of IBD research. Eventually, genetic research may be able to classify different disease phenotypes on a more detailed molecular basis and may provide important contributions in the development of new therapeutic approaches.
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