REVIEW

Novel susceptibility genes in inflammatory bowel disease

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Abstract

The inflammatory bowel disease, Crohn's disease and ulcerative colitis, are polygenic disorders with important environmental interactions. To date, the most widely adopted approach to identifying susceptibility genes in complex diseases has involved genome wide linkage studies followed by studies of positional candidate genes in loci of interest. This review encompasses data from studies into novel candidate genes implicated in the pathogenesis of inflammatory bowel disease. Novel techniques to identify candidate genes-genome wide association studies, yeast-two hybrid screening, microarray gene expression studies and proteomic profiling, are also reviewed and their potential role in unravelling the pathogenesis of inflammatory bowel disease are discussed.

INTRODUCTION

Following the identification of the NOD2/CARD15 gene in 2001[1,2], the impetus has grown to identify novel genetic determinants of susceptibility and phenotype in the inflammatory bowel diseases, Crohn's disease and ulcerative colitis. The inflammatory bowel disease are now considered to be non-Mendelian polygenic disorders with important environmental interactions[3], and as such the accepted approach to identifying candidate genes in complex disease genetics has been adopted, namely genome wide scanning followed by studies of positional candidate genes in sub-chromosomal regions of association (loci).

A number of candidate genes and susceptibility loci have been discussed elsewhere in this issue, so in this review we first summarise recent data with regards to genome wide scanning and candidate gene studies, which may lead to gene identification. Novel techniques to identify candidate genes - genome wide association studies, yeast- two hybrid screening, microarray gene expression studies and proteomic profiling, are subsequently discussed.

GENE FINDING APPROACHES

Genome wide scan approach

Genome-wide linkage analysis using highly polymorphic microsatellite markers identified during the course of the Human Genome Project has led to success in identifying genetic determinants both in single gene disorders and in complex genetic diseases. To date, successful genome scans have typically involved several hundred microsatellite markers and a large number of multiply affected inflammatory bowel disease families (typically sibling pairs), with the aim of examining whether the degree of sharing variant alleles between affected individuals exceeds that as expected by chance alone[4].

To date, fourteen genome wide scans have been carried out in patients with inflammatory bowel disease. The most widely accepted guidelines for assessing the results are those defined in 1995 by Lander and Kruglyak who proposed criteria for reporting areas of linkage[5], with areas of 'suggestive linkage' having LOD scores of 2.2 and above and P values of less than 7×10^{-4}, areas of 'significant linkage' having LOD scores above 3.6 and P values of less than 2×10^{-5}, and areas of 'highly significant linkage' having LOD scores of 5.4 and above and P values of less than 3×10^{-7}. Areas of' confirmed linkage defined as areas of significant linkage have been replicated in an independent cohort, with a nominal P value of less than 0.01. Using these criteria, loci with confirmed linkage have been identified on chromosomes 1[6], 3 (IBD9)[7,8], 5 (IBD5)[9-11], 6 (IBD3; HLA)[10,12], 12 (IBD2)[13], 14 (IBD4)[9,14], 16 (IBD1)[13,15] and 19 (IBD6)[10] (Figure 1).

These confirmed regions of linkage need to be further narrowed by fine mapping of these areas, as each spans a large genomic region. The classical positional cloning approach is used by Hugot and colleagues to identify NOD2/CARD15 as the gene confers the critical mutations in IBD1 on chromosome 16[1]. Alternative strategies for fine mapping these regions or providing a short-cut to the critical genes are discussed in the following.
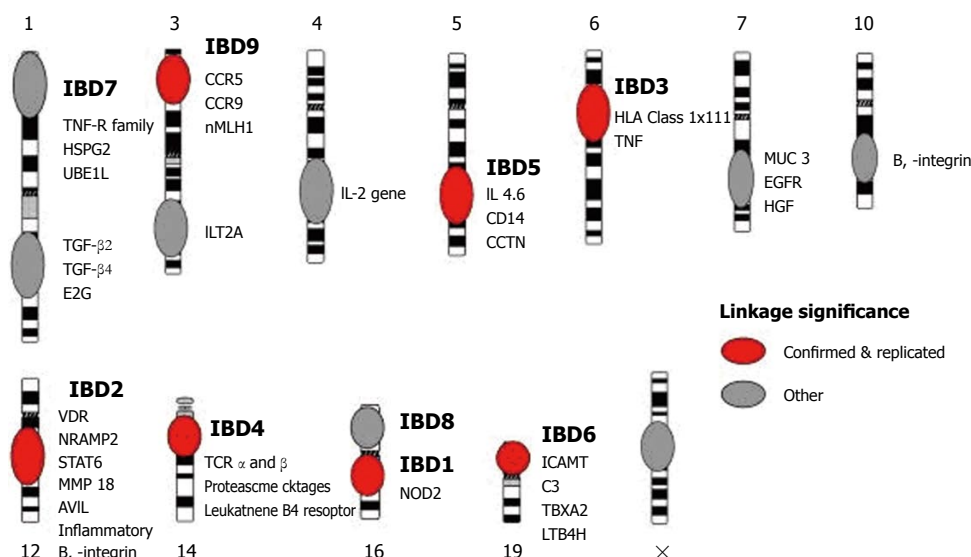
Figure 1 Confirmed and suggested linkage intervals from genome wide scans in inflammatory bowel diseases with the potential candidate genes coloured in red.

Significance levels defined by Lander and Kruglyak [95].

Positional candidate gene approach

The positional candidate gene approach to identifying association involves the use of data concerning the chromosomal location of the candidate gene as well as functional or expression data. In inflammatory bowel disease genes involved in the regulation of the innate immune system, mucosal integrity and cell-cell interactions are all clearly plausible candidate genes. By comparing the allelic frequencies of variants in these genes between patients with IBD and matched controls (case control analysis), or by investigating intra-familial association/ linkage, genes containing critical disease causing mutations may be identified.

Using this approach, Ogura and colleagues[2] identified the frameshift mutation in the NOD2/CARD15 gene (Leu1007fsinsC). Stoll and colleagues[16] also identified the DLG5 (Drosophila Discs Large Homolog 5) gene located on chromosome 10q23 by candidate gene approach allied to its position as being associated with inflammatory bowel disease in the German population[16].

However, whilst these approaches have had notable success, especially with the identification of the NOD2/CARD15 gene within the IBD1 locus, there have been difficulties in identifying candidate genes within other loci, notably the IBD5 locus as discussed elsewhere. Due to the tight linkage disequilibrium across the IBD5 locus and the wealth of candidate genes in the region[17], it has been impossible to identify with certainty the causative mutations in this region. Although Peltekova and colleagues[18] have suggested that the OCTN1/2 genes contain the causative mutations, considerably larger cohorts of inflammatory bowel disease patients (around 3000 patients) are needed to confirm these findings using genetic studies alone and functional and expression data can help delineate this region[19].

NOVEL GENES AND LOCI CURRENTLY UNDER INVESTIGATION

Genes involved in initiation and regulation of the innate immune system

The function of the immune system is to be able to rec-ognise the vast number of antigens that are present in our environment, to mount an appropriate response to invasive pathogens and also to limit damage during inflammation and immune response to these pathogens. Dysregulation of the immune system can lead to immune-mediated diseases such as inflammatory bowel disease. Following the discovery of NOD2/CARD15, attention has focussed on the innate immune system[20]. From an evolutionary prospective, the innate immune response is more ancient than the adaptive immune response present in nearly all taxa[21]. As a further measure of the importance of the innate immune response in plants, genes involved in plant disease resistance occupy >1% of the genome of primitive species, such as *Rockcress*[22].

Toll like receptors (TLRs) are transmembrane proteins which play a pivotal role in mediating the innate immune response to viral, bacterial and fungal pathogens[23]. TLRs were initially identified in *Drosophila* and they are widely conserved across animal species[24]. In mammals TLRs are integral membrane glycoproteins which recognise conserved products unique to microbial metabolism and signal via a number of downstream signalling molecules (MyD88, Il-1R-associated kinases, TGF-β, and TNF- receptor associated factor 6)[25]. To date 11 members of the TLR family have been identified in mammals[24].

TLR4

TLR4 functions as a sensor for lipopolysaccharide (LPS), part of the cell membrane of Gram negative bacteria[26], and as such has been considered a strong functional candidate gene in the pathogenesis of inflammatory bowel disease as well as in other immunoinflammatory diseases. Variant alleles of the TLR4 Asp299Gly polymorphism have been associated with decreased bronchial responsiveness to LPS in humans and reduced activation in transfection experiments[27]. The Asp299Gly polymorphism has been associated with Crohn's disease and ulcerative colitis both in a cohort of Belgian patients[28] and in cohorts from Greece[29] (Table 1).

An association was observed between ulcerative colitis

Table 1 Number of patients studied and Toll-like receptor 4 Asp299Gly allele frequency (as a percentage of each group) in healthy controls, ulcerative colitis and Crohn's disease patients			
	Healthy controls (%)	Ulcerative colitis (%)	Crohn's disease (%)	*P* value for controls versus Crohn's disease (NS: not significant)
Franchimont *et al*, Belgium (28)	139 (5.0)	163 (10.0)	334 (11.0)	0.007
Arnott *et al*, Scotland (32)	189 (8.8)	246 (6.8)	234 (10.3)	NS
Torok *et al*, Germany (30)	145 (4.0)	98 (9.0)	102 (7.0)	NS
Gazouli *et al*, Greece (29)	100 (3)	85 (3.5)	120 (7.9)	0.026
Brand *et al*, Germany (31)	199 (7.5)		204 (14.2)	0.038
Oostenbrug *et al*, Netherlands (33)	296 (4.6)	179 (5.9)	393 (6.7)	NS

These data from 6 European case control studies looking at the allelic frequencies of the Asp299Gly polymorphism of the TLR4 gene illustrate the contrasting results from these studies, and furthermore suggest a degree of genetic heterogeneity between the different populations.

and the TLR4 Thr399Ile polymorphism but not the Asp299Gly variant in one German cohort[30]. However, in a further cohort of German patients Asp299Gly variants were associated with Crohn's disease[31]. No association between inflammatory bowel disease and the TLR4 Asp299Gly polymorphism has been observed in cohorts of patients from Scotland[32]. In the light of these discordant data-sets, it is difficult to ascertain whether these two polymorphisms represent the critical disease causing mutations in the TLR4 gene[33]. Recent data from two independent mouse models have shown that treatment with TLR4 antagonists may prevent the development of colitis, further suggesting that this innate immune receptor may be central to the pathogenesis of inflammatory bowel disease[34].

TLR5
TLR5 functions by recognising flagellin, a common bacterial antigen that is present in enteric bacteria[35, 36]. Strong serological response to flagellin in multiple animal models of colitis has been observed by Lodes and colleagues[37], who demonstrated that colitis can be induced by transferring flagellin-specific T cells to immunodeficient animals. Further evidence implicating TLR5 polymorphisms in the pathogenesis of inflammatory bowel disease suggests that carriage of a dominant negative TLR5 polymorphism (TLR5-stop) appears to protect against Crohn's disease and results in a significant reduction in flagellin-specific circulating concentrations of IgA and IgG[38]. These data linking a genetic defect in TLR5 to an alteration in the acquired immune response are intriguing and of great pertinence as recent studies report synergism between NOD2/CARD15 and TLR5 signalling[39].

NOD-LLR protein family
NOD (nucleotide- binding oligomerization domain) - LLR (leucine rich region) proteins are part of the CATERPILL-ER (CARD, transcription enhancer, R [purine]- binding, pyrin, lots of purine repeats) family, and these proteins are key regulators of innate immunity and apoptosis in mammals and plants[40]. NOD- LLR proteins play a central role in recognising pathogens on the cell surface and in the cy-

tosol, and a family of greater than twenty human proteins possessing a NOD domain has been identified[41]. NOD-LLR proteins are comprised of three distinct functional domains: the amino terminal effector domain involved in signalling, the centrally located NOD domain and the LLR ligand-sensing domain[42]. Three of the human NOD-LLR proteins have effector domains, namely caspase-recruitment domains (CARD): NOD1/CARD4, NOD2/CARD15 and Apaf-1.[40]

With the established role of NOD2/CARD15 in the pathogenesis of Crohn's disease, attention has focused on NOD1/CARD4 identified by two groups in 1999[43,44]. NOD1/CARD4 has a similar structure to NOD2/CARD15 with it having only one CARD domain, a central NOD domain and a LLR[45]. NOD1 detects intracellular diaminopimelic acid, a tripeptide motif found in many Gram negative bacteria and unique to Gram negative proteoglycans[46,47]. NOD1/CARD4 plays a role in colonic epithelial defence against the intracellular pathogens *E. coli* and *Shigella flexeneri*[48,49]. Its effector domain is associated with RipK2(a CARD-containing interleukin-1 beta-converting enzyme-associated kinase), thus mediating NFκB activation[43,44,50].

Linkage to the area that encompasses the NOD1/CARD4 gene, chromosome 7p14, was first found on a genome wide scan of the UK population in 1996[13]. This initial finding is replicated in two further genome wide scans involving patients in North America (USA and Canada)[6,10]. In a pan-European study involving 381 inflammatory bowel disease families from France, Sweden, Belgium, Spain, Denmark, Italy and Ireland (235 CD and 58 UC), no association between susceptibility to IBD and the E266K variant which lies within the CARD domain of NOD1/CARD4 was observed[51].

However, a further recent UK study of 556 IBD trios (294 CD and 252 UC) a significant association was observed between a complex insertion deletion allele (CCCCCCAC/ CCCCCCCCC) of NOD1/CARD4 at nucleotides 30, 258, 950 (Ensembl build 35) which is partially identified by rs6958571 and is located within intron 9, and predisposition to inflammatory bowel disease ($P = 0.002$)

and, also to ulcerative colitis ($P = 0.01$)[52]. By creating a sliding two marker haplotype using this insertion deletion allele and the rs2907748 (T → C) variant at nucleotides 30,246,263, the association was strengthened with P values of 3×10^{-6} for inflammatory bowel disease, 7×10^{-4} for Crohn's disease and 3×10^{-5} for ulcerative colitis. The deletion allele was significantly associated with an early age of onset of IBD (<25 years) and there was no evidence of an epistatic effect when NOD1/CARD4, NOD2/CARD15 and IBD5 are investigated.

Interestingly allelic variation of the same insertion deletion polymorphism (CCCCCCAC/ CCCCCCCCC) at nucleotides 30, 258, 950, which appear to alter the protein binding of NOD1/CARD4, is also associated with susceptibility to childhood onset of asthma and elevated serum IgE levels[53]. Further genetic and functional data with regards to NOD1/CARD4 are required to discover whether these variants play a role in the pathogenesis of inflammatory bowel disease in racially diverse populations.

CCL20

CC chemokine ligand (CCL) 20 is responsible for the chemoattraction of immature dendritic cells (DC) expressing the CCR6 receptor in the epithelium and Peyer's patches in the bowel mucosa[54]. The ligand- receptor pair of CCL20 and CCR6 also play a role in the chemoattraction of effector/memory T-cells and B-cells under homeostatic and inflammatory conditions, in diseases including cancer and rheumatoid arthritis[55]. Microarray and real-time PCR analysis of endoscopic colonic biopsies of patients with IBD have revealed increased levels of CCL20 mRNA in histologically inflamed biopsies when compared to non-inflamed IBD biopsies and inflamed non-IBD colonic biopsies[56,57].

Using microarray analysis of peripheral blood mononuclear cells in 8 patients with ulcerative colitis and 8 healthy controls, Choi and colleagues showed that the CCL20 gene is upregulated in ulcerative colitis samples[58]. Five SNPs in the promoter region of CCL20 are identified and analysed in a case control study of 118 ulcerative colitis patients and 300 healthy controls in the Korean population. Three of the CCL20 variants are significantly associated with ulcerative colitis ($P < 0.0038$) and further replication data in ethnically diverse cohorts of patients with IBD are required.

Other loci

The identification of other genetic variants in loci identified by genome wide scan continues to yield other potential determinants of inflammatory bowel disease. These genes may play a role that is limited to specific populations and disease phenotypes. However, their identification can unravel the pathogenesis of inflammatory bowel disease and may reveal new therapeutic targets.

Other loci of current interest include the IBD2 locus on chromosome 12 first described in the UK[13] and subsequent analysis suggests that this locus is most strongly associated with colonic disease[59]. In the Flemish population the IBD4 region on chromosome 14 appears to be an important determinant of Crohn's disease[60] and this association has been replicated in the USA[14] and by the International IBD genetics consortium[61]. Fine mapping of the IBD2 and the IBD4 loci are required to establish which genes in these regions contain critical disease causing mutations.

NOVEL TECHNIQUES TO IDENTIFY CANDIDATE GENES

Genome wide association studies

With the advent of the HapMap project[62] and plans to include information on around 300 million genotypes, genome-wide association studies have been proposed as a powerful tool in identifying common variants that contribute to complex genetic traits[63]. Genome wide association studies compare the frequency of alleles and genotypes between cases and controls on a genome-wide scale, thus creating a comprehensive unbiased method to identify candidate genes. Advances in technology lowering the price of genotyping on a large scale and genome wide linkage disequilibrium mapping using data from the HapMap project have made this method available. However, significant concerns remain about the power of these studies and it has been proposed that levels of significance of $P < 5 \times 10^{-8}$ are required to constitute significance in genome wide association studies[64].

Using this approach Tamiya and colleagues[65] placed 27 039 microsatellite markers across the human genome at 100kb intervals in 470 patients with rheumatoid arthritis and 470 controls. Forty seven candidate regions were identified and the previously implicated major histocompatibility complex gene HLA- DRB1 in the chromosomal region 6p21.3 was shown to be associated with rheumatoid arthritis. In the UK，the Wellcome Trust has provided initial funding for a phase I study including 1 000 patients with Crohn's disease and 3 000 controls, 675 000 SNPs will be genotyped to cover the entire genome and results are expected in 12 months.

Yeast two- hybrid screening

The yeast two-hybrid assay is an elegant means of investigating protein-protein interactions, which have become increasingly important in our understanding of biological systems and pathways. The yeast two- hybrid model can also be used to characterize interactions already known to occur, thus helping delineate the protein domains responsible for interaction and the environmental conditions involved[66].

The yeast two-hybrid assay is performed in the budding yeast, *S cerevisiae* using two fusion proteins: the target protein of interest, known as 'bait' is fused to a DNA binding domain attached to its N-terminus. The second protein, the 'prey' is fused to an activation domain. If the bait protein interacts with the prey, these bring the binding domain and the activation domain of transcriptional activator together, which in turn switches on the expression of the reporter genes. The reporter genes are constructed to allow growth of the yeast in a selective medium when the interaction occurs. When investigating 'protein- protein' interactions, a single bait protein is used to search for interaction with a library of proteins fused to the activation domain. The choice of library is determined

by the tissue of interest, e.g. intestinal cell library for inflammatory bowel disease[67].

Schizophrenia is a disease of polygenic genetic susceptibility where the yeast two hybrid systems have been successfully used to identify candidate genes. The disrupted-in- schizophrenia 1 (DISC1) gene was identified in 2 000[68] and confirmed in other cohorts,[69,70] was used as bait. DISC1 encodes a novel protein of unknown function and full-length human DISC1 protein was used to screen human adult and foetal brain libraries for interacting proteins, using the yeast two-hybrid system[71]. Twenty-one proteins from a variety of locations have been identified implicating DISC1 in several aspects of central nervous system signalling and confirming data from other yeast two- hybrid scans using DISC1 as bait[72,73]. From these data the authors are able to identify a number of potential DISC1 interactions and to speculate that DISC1 may be at the centre of an extensive protein interaction network.

Yeast two- hybrid as an investigative tool in inflammatory bowel disease

Barnich and colleagues[74] used NOD2/CARD15 gene as 'bait' to screen a bone marrow library and identified GRIM19, a protein with homology to the NADPH dehydrogenase which interacts with endogenous NOD2[74]. GRIM19 is required for NFκB activation following NOD2-mediated recognition of bacterial muramyl dipeptide and the authors hypothesised that GRIM19 is a key component of the CARD15/NOD2 signalling pathway which currently remains under detailed investigation[75-77]. In our own protein yeast two- hybrid studies a series of 12 candidate genes which interact with CARD15/NOD2 (*Nimmo, Satsangi,* unpublished data) have been identified.

GENOMICS

Microarray expression studies

Gene expression technology using microarray allows a comprehensive picture of gene expression at the tissue and cellular level, thus helping understand the underlying physiological and pathological processes. Microarray technology has developed from spotted nylon array technology used to identify genomic inserts in bacterial colonies by hybridisation with preidentified cDNAs[78]. In the seminal microarray experiment in 1995, a two-colour fluorescent pattern of differential gene expression is generated when comparing 48 genes in the root and the shoot of *Arabidopsis*[79].

Since 1995 there has been a rapid increase in the number of papers published using microarray technology from 7 in 1995-1996 to 139 in 1999 and to 3 000 in 2003[80]. The initial optimism set out by Mark Schena, one of the authors of the *Arabidopsis* microarray experiment suggesting that all human diseases can be studied by microarray technology, With the ultimate goal of this work to develop effective treatments and cures for every human disease by 2 050 has not yet fully been born out by the published data. However, a number of exciting and novel observations have been generated.

Microarray design

RNA isolated from the tissue sample or cells can be used to generate cRNA or cDNA[78]. The probe set is then designed with transcripts targeting the genes of interest and care needs to be taken to prevent cross- hybridization. It is worth noting at this point that the probe refers to the reporter sequence placed at a particular position on the array which interrogates the sample and not the other way around. The probes are hybridised on the chip with the target unknown cRNA or cDNA. Following this process which can take several hours, the unbound target is washed off and the arrays are fluorescently labelled, so that they can be analyzed by confocal laser scanning. Expression of the entire human genome can be analyzed on one chip and complex computational and statistical techniques have been developed to analyze expression data because of the large amount of data microarrays[81].

Clinical data generated by microarray

Probably the most provocative and clinically relevant data generated by microarray have been in the field of cancer research. An example of the potential of microarray has demonstrated by Alizadeh and colleagues[82]. By comparing expression analysis on a 'lymphochip' panel of 3186 genes between patients with diffuse large B- cell lymphoma (DLBCL) prior to treatment, the investigators are able to group these patients into two discrete groups: germinal centre DLBCL and activated DLBCL. When the clinical progress of these two groups of patients were examined, the germinal cell DLBCL had a higher five year survival rate than the activated DLBCL (75% $n = 25$ versus 16% $n = 37$ respectively, $P \leqslant 0.01$). Gene expression profiles have also been successfully used to predict prognosis in 295 patients with breast cancer[83]. However, a meta- analysis of 84 studies found that DNA microarrays have a variable performance in measuring prognosis in a number of different cancers[84].

Microarray experiments in subjects with inflammatory bowel disease

Microarray has been used to compare synovial tissue obtained from patients with severe rheumatoid arthritis and macroscopically affected bowel of patients undergoing surgery for Crohn's disease[85]. A number of inflammatory genes were commonly expressed in diseased tissues. In 2000 Dieckgraefe and colleagues[86] published a study using microarray technology to compare patients with ulcerative colitis undergoing colectomies for disease refractory to medical management and a control group. Six thousand five hundred genes were analysed and the results confirmed an increase in a number of genes previously implicated in the pathogenesis of ulcerative colitis (IL-1, IL-1 RA and IL8) and suggested that multiple members of the chemokine subfamily may play a role in disease pathogenesis.

In a further microarray experiment using surgically resected inflammatory bowel disease tissue, 7 070 genes were examined and 170 genes were differentially expressed in ulcerative colitis and Crohn's disease with almost an equal number up regulated and down regulated[87]. Twenty percent of the differentially regulated genes were common to both forms of inflammatory bowel disease and when the locations of these genes were mapped several were found to lie within IBD2 locus on chromosome 12.

More recently Langman and colleagues[88] used micro-array to analyze biopsies taken from patients with Crohn's disease, ulcerative colitis and control patients, 22 283 genes were analysed. They found that genes involved in cellular detoxification and biotransformation (pregnane X receptor and MDR1) are significantly down regulated in the colon of patients with ulcerative colitis.

Endoscopic investigation of inflammatory bowel disease with the ability to take pinch mucosal biopsies has allowed investigators to take microarray tissue from a larger range of patients including those with less severe disease compared to patients requiring rescectional surgery. In a study of 24 patients with ulcerative colitis, Okahara and colleagues[89] investigated the difference in gene expression between endoscopic biopsies taken from inflamed and non inflamed areas using a 1300 gene microarray, and found that migration inhibitory factor- related protein 14 (MRP14), growth-related oncogene gamma (GROγ) and serum amyloid A1 (SAA1) were upregulated whereas TIMP1 and PDZ and LIM domain 1 (elfin) were down regulated in the inflamed biopsies when compared to the non- inflamed biopsies.

In a study involving endoscopic biopsies of patients with Crohn's disease, ulcerative colitis and controls, Costello and colleagues[90] found that 500 and 272 transcripts are differentially regulated in CD and UC, respectively. Candidate genes are confirmed by real-time PCR and immunohistochemistry, and a number of genes involved in immune regulation were identified.

Further microarray studies in inflammatory bowel disease

Microarray has been useful in studies thus far and continues to be a powerful tool in investigation of the pathogenesis of inflammatory bowel disease. Further studies need to be designed in order to allow the collection of accurate clinical data describing the phenotype and activity of inflammatory bowel disease at the time of sample collection as these data are critical in analysis and interpretation of the results. The confounding problems associated with the heterogeneous mixture of cells, which is inevitable in the analysis of entire biopsy samples, can be reduced by using laser capture micro-dissection, in order to analyze only the cells of interest. By obtaining painstakingly accurate clinical information at the time of biopsy collection and narrowing down cell heterogeneity, the amount of background 'noise' that has hampered previous microarray studies can be reduced[91]. Minimum standards of information reported on microarray data have been proposed with the aim of establishing a standard for recording micoarray-based gene expression data[92], and replication of results with real-time PCR helps to validate these studies.

PROTEOMICS

Proteomic analysis is another relatively new investigative tool that has not been used to any large extent in the field of inflammatory bowel disease. Proteomics refers to the study of the total protein content in cells and the products of genes, so as to identify differences between normal and diseased tissue[93]. This is achieved by combining the techniques of protein electrophoresis and mass spectrometry. Expression profiles of proteomes may be generated from

samples of serum or secreted fluid, and may be able to differentiate disease progression, response to therapy and to identify novel therapeutic targets.

Preliminary data have been generated in our unit by comparing serum of patients with severe ulcerative colitis who responded to corticosteroid therapy and matched patients who were resistant to and failed corticosteroid therapy[94]. Proteomic profiles of corticosteroid resistant and responsive groups are significantly different at 19 protein biomarkers: 12 proteins were up-regulated and 7 proteins were down-regulated in the corticosteroid resistant group. These results suggest that protein profiling may be useful in predicting patient response to corticosteroid therapy and identification of these proteins is currently underway.

CONCLUSION

The application of novel technologies has catalyzed the search for novel determinants in inflammatory bowel disease. Proof of the principle for genome wide studies is provided by the NOD2/CARD15 discovery and investigation of genes at other loci is underway. Rigorous attention to statistical design and phenotypic classification of disease is of paramount importance in gene discovery.

With the advent of new powerful investigative tools such as microarray and proteomic analysis, large amounts of data can be generated from small studies in inflammatory bowel disease. Among the clinical challenges in harnessing the power of these new investigative tools will be to accurately define clinical phenotype in patients studied, given the heterogeneity inevitable in these studies.

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