Genetics of inflammatory bowel disease: from bench to bedside?

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Epidemiological data, particularly concordance rates in twin pairs and in multiply affected families, provide strong evidence that both genetic and environmental influences are important in the development of the chronic intestinal inflammation characteristic of Crohn disease and ulcerative colitis. Furthermore, supplementary data now suggest that not only susceptibility, but also disease behavior and response to therapy may have a strong genetic influence. The model of disease susceptibility most pertinent to the inflammatory bowel diseases is that Crohn disease and ulcerative colitis are related polygenic disorders. Recently, this model has received strong support from the results of genome-wide scanning and candidate gene studies carried out in European, North American and Australian populations. In spite of all potential difficulties related to disease and ethnic heterogeneity, consistent replication of linkage has been found with distinct regions on chromosomes 16, 12, 6 (the major histocompatibility complex) and most recently chromosome 14. Whereas the linkages on chromosome 16 and 14 appear strongest in Crohn disease, the chromosome 12 locus appears most pertinent to ulcerative colitis, and the HLA region appears more pertinent in all forms of inflammatory bowel disease. The current challenge is to narrow these regions of linkage and identify the susceptibility genes within the regions. The task may be greatly benefited by the recent successful sequence data available from the human genome project. Compelling data have emerged to suggest genetic markers that may allow prediction of disease severity, and efficacy and tolerability of immuno-suppressants commonly used in inflammatory bowel disease. □ *Chromosomal linkage; epidemiology*

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The chronic inflammatory bowel diseases, Crohn disease and ulcerative colitis, are now common causes of gastrointestinal morbidity in Western Europe and North America. Population-based studies in the United Kingdom suggest a disease prevalence of between 300 and 400/100,000 population (1). These illnesses most commonly present in teenage or early adulthood, and the natural history is of chronic relapsing illness with periods of remission, interrupted by exacerbations. In spite of the many potential toxicities, corticosteroids and immunosuppressants have remained the mainstay of medical therapy for active disease. Surgery is necessary for disease that is medically unresponsive or for complications.

Crohn disease may affect any part of the gastrointestinal tract, and is characterized by discontinuous areas of transmural inflammation. Ulcerative colitis affects the large bowel only, and inflammation may characteristically be limited to the colonic mucosa. However, both diseases can be complicated by similar extra-intestinal manifestations (affecting eyes, skin, and joints) or by colorectal malignancy. Furthermore, in 10% of cases of inflammatory bowel disease affecting the colon, the diagnosis of 'indeterminate colitis' is made, as differentiation between Crohn disease and ulcerative colitis is not possible. Both illnesses may occur in the same family more frequently than expected by chance alone.

In recent years, real progress has been made in understanding the pathogenesis of these disabling illnesses. Consistent epidemiological data implicate both genetic and environmental influences in disease pathogenesis. The familial clustering, particularly concordance rates in twin pairs and in siblings, have emphasized the strength of the genetic contribution, which is particularly marked in Crohn disease. Three recent European studies have reported concordance rates in a total of 322 twin pairs from Sweden (2), Denmark (3), and the United Kingdom (4). The overall concordance rates in Crohn disease were 37% and 7% for monozygotic and dizygotic twin pairs, respectively; the corresponding results in ulcerative colitis were 10% and 3%. The coefficient of heritability derived from these data quantifies the genetic contribution in these illnesses. In Crohn disease the derived values for heritability suggest that the genetic contribution is greater than in many common illnesses, including schizophrenia, hypertension, and equivalent to that seen in insulin-dependent diabetes or multiple sclerosis (2).

Familial clustering may best be expressed as the ratio of disease risk to siblings of patients with inflammatory bowel disease, compared with the general population risk (λs). This value is of particular benefit to geneticists in assessing the feasibility of genome-wide scanning techniques in detecting susceptibility genes. This ratio (λs) is documented as between 15 and 35 in Crohn disease and between 7 and 15 in ulcerative colitis. By contrast, λs is approximately 15 in insulin-dependent diabetes and only 2 in hypertension (5).

Further evidence for the importance of genetic susceptibility in inflammatory bowel disease is derived from reported differences in disease susceptibility in ethnic groups. Clear differences have been reported, with the Ashkenazi Jewish population at greatest risk of both inflammatory bowel disease and familial inflammatory

bowel disease. Although migrant studies, particularly those involving Asians in the United Kingdom, suggest that environmental factors may play a part in determining these ethnic differences in inflammatory bowel disease prevalence, it is noteworthy that the Ashkenazi Jewish population has been particularly prone to many single gene disorders, and that the linkage data in inflammatory bowel disease to date do suggest a strong distinct genetic contribution in this ethnic group.

Model of disease inheritance

Although complex segregation analyses in Europe have suggested that a single gene may be important in Crohn disease (recessive inheritance) (6) and ulcerative colitis (dominant inheritance) (7), the genetic epidemiological data presented above are not consistent with a simple Mendelian mode of inheritance in inflammatory bowel disease. The model that appears most consistent with epidemiological data, and with the clinical variability in presentation, is that Crohn disease and ulcerative colitis represent related polygenic disorders which may share some but not all susceptibility genes. In defining this model, the variability of clinical presentation of disease must be borne in mind. Both Crohn disease and ulcerative colitis are variable in clinical presentation, in terms of disease site, extent, response to therapy and extra-intestinal manifestations. Gene–gene interaction, gene–environmental interaction, and allelic and locus heterogeneity may all contribute to the clinical phenotype of the disease (8).

The resulting model is therefore of complex polygenic disorders, with considerable disease heterogeneity. The model proposes a hierarchy of influence in inflammatory bowel disease, with both disease-specific genetic genes and phenotype determining genes of importance.

Searching for genetic influences in inflammatory bowel disease

In common with the majority of human diseases, and indeed behavioral traits, the inflammatory bowel diseases are therefore regarded as complex, with the clinical presentation reflecting the interaction between genes and environment. It is only in recent years that identification of the genetic determinants in complex traits has become feasible. Two complementary approaches have been employed, and it is likely that these approaches, along with expression studies and studies of gene function, will lead to definitive identification of genetic determinants in inflammatory bowel disease in the near future.

Scanning the entire human genome for genetic determinants in complex diseases is now a realistic goal for scientists involved with many common disorders. Initial progress was most rapid in studies of insulin-dependent diabetes, and two landmark publications in 1994 served as conclusive proof of principle of this technique (9, 10). Two years later, the first studies involving multiply affected families with inflammatory bowel disease were published. The European collaborative group led by Giles Thomas and Jean-Pierre Hugot (Paris) used a two-stage approach involving affected sibling pairs with Crohn disease only (11). In the first stage, 40 sibling pairs (25 families) were studied. Allele sharing patterns in these sibling pair families demonstrated preliminary evidence for linkage with a region in the pericentromeric region of chromosome 16 ($P < 0.01$). The investigators then studied a second set of 70 sibling pairs, and were again able to demonstrate distortion of allele sharing in the pericentromeric region of chromosome 16. The authors designated this locus, IBD1, and estimated that the sibling relative risk attributable to the locus was 1.3.

Considerable reservations were expressed regarding the size of this study, and the relatively small effect of the locus on chromosome 16 identified. Nevertheless, the investigators' initial observations have now been entirely vindicated. Positive replication studies have been reported widely in Crohn disease populations from Europe, North America, and Australia (12–16). The data from Australia are particularly compelling, producing very strong linkage data for this region. Moreover, a consortium of international investigators formed initially in 1997 have now been able to pool data from 11 centers throughout the world, and to confirm the importance of the IBD1 region in Crohn disease susceptibility (17).

The second genome-wide scan reported in inflammatory bowel disease in 1996 involved 186 sibling pairs for 160 nuclear families (18). These authors included 81 sibling pairs in which both children had Crohn disease, 64 in which both siblings had ulcerative colitis and 41 mixed pairs. The authors employed a two-stage approach, and were able to provide strong evidence for the presence of susceptibility locus on chromosome 12 ($P = 2.66 \times 10^{-7}$). In the initial publication, this chromosome 12 locus, subsequently designated as IBD2, appeared to be pertinent in both Crohn disease and ulcerative colitis. Subsequent analyses by the same group, and investigators in Pittsburgh suggest that the IBD2 locus may in fact be more influential in ulcerative colitis than in Crohn disease (19). Evidence for replication in independent populations for the IBD2 locus has been less strong than for IBD1—this may well reflect that the majority of replication studies have involved data sets dominated by Crohn disease families. Nevertheless, even using the stringent criteria suggested by Lander and Kruglyak in their landmark paper, this locus does show confirmed evidence for linkage.

The investigators in Oxford also identified a number of other loci which demonstrated a lesser degree of linkage evidence. In particular, loci on chromosome 7 and 3 were suggested to be involved in inflammatory bowel disease, whereas loci on chromosome 2 and 6 (the HLA region) were suggested to be loci with an effect only on susceptibility to ulcerative colitis. In a subsequent paper

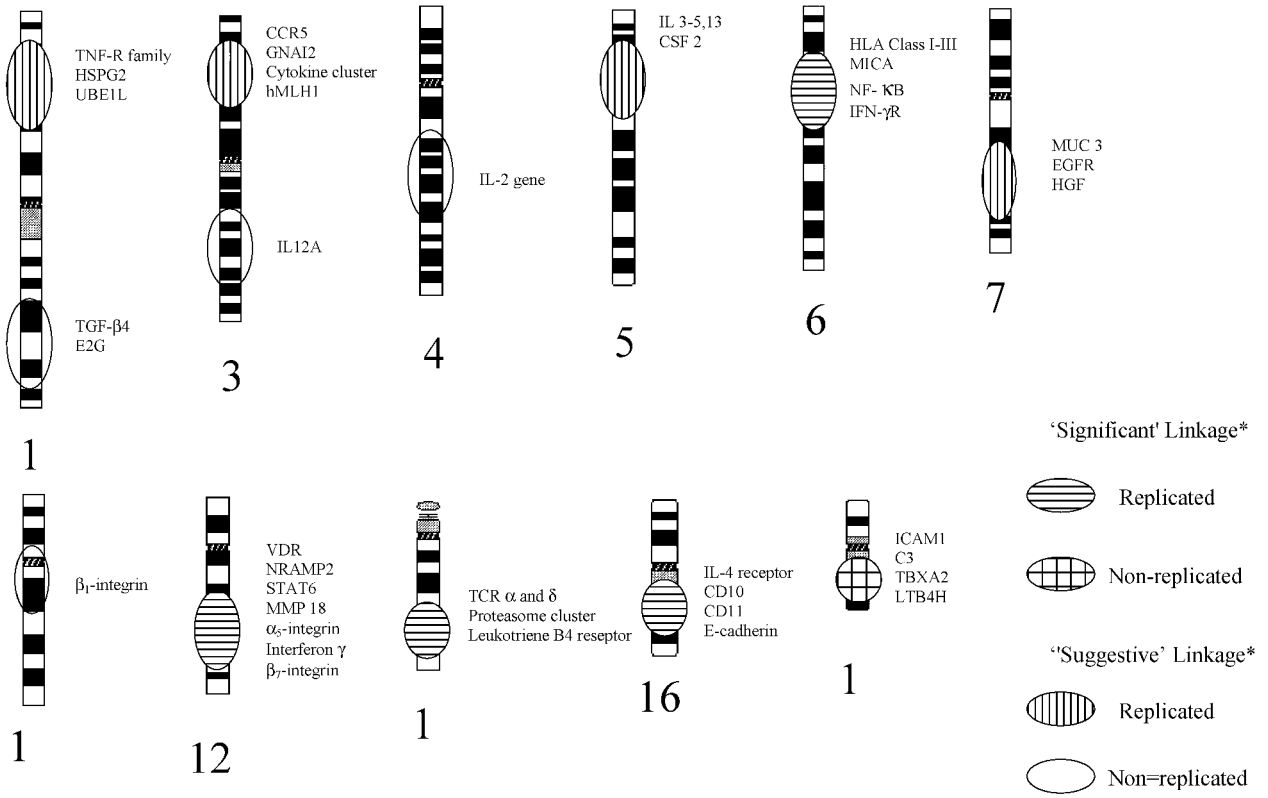

Fig. 1. Linkage regions and positional candidate genes in IBD.

the same investigators confirmed the IBD1 locus in Crohn disease and suggested that these data have provided the most compelling evidence for the model of Crohn disease and ulcerative colitis as related polygenic diseases (12).

In the 5 years since those initial publications, there has been considerable rapid progress using the technique of genome-wide screening in inflammatory bowel diseases. Other investigators in Europe, and in the United States have published the results of full genome-wide scans, and replication studies (20–25). As the difficulties involved in the techniques have become fully appreciated, all aspects of study design have also evolved. In particular, robotic technology is now available for rapid fast through-put genotyping, and statistical analysis packages may be accessed freely. The quality of the publications has reflected this improvement in expertise and analysis techniques: in particular, the publications from Schreiber's group in Kiel, and the highly productive U.S. collaborative groups in Baltimore and Chicago have demonstrated the continuing strengths of the international research groups.

A large number of chromosomal subregions have been implicated in these subsequent genome scans from Europe and North America. Of these, two further regions appear to have been replicated with confidence. The HLA region on chromosome 6p has long been a candidate susceptibility region in inflammatory bowel disease. Early studies, involving the candidate gene approach, had yielded inconclusive results, probably due to a combination of inadequate power, poor genotyping, and the confounding factors of ethnic and disease heterogeneity. However, linkage studies and association studies carried out using modern molecular genotyping methods provide strong evidence that genes within the HLA region are important determinants of susceptibility and disease behavior in inflammatory bowel disease. Satsangi et al. (26) carried out a detailed study involving both non-parametric linkage analysis and allelic association study in the UK population. These data suggested that the HLA region was more influential in ulcerative colitis than in Crohn disease, and identified specific allelic associations with severe disease and extra-intestinal manifestations.

Subsequent analysis in Europe and North America confirmed linkage and the allelic associations with the HLA DRB1*0103 allele (27, 28) (severe, extensive colitis) were also sustained. The more recent linkage data implicate the genes of the HLA region in both ulcerative colitis and Crohn disease, although the primary susceptibility gene remains under investigation. This region is now identified as IBD3. Stokkers et al. (29) have provided a valuable mega-analysis of 29 HLA studies between 1966 and 1998 in inflammatory bowel disease.

Most recently, data from Pittsburgh, Los Angeles (25), and Leuven (30) suggest the presence of a distinct

susceptibility locus in Crohn disease on chromosome 14, and this region has been labeled IBD4.

Beyond linkage studies?

In addition to the four replicated regions of linkage described above, a number of other sub-chromosomal regions have been implicated to a greater or lesser extent in the recent studies (see Fig. 1). There has been considerable discussion amongst clinicians and geneticists as to which of the documented linkages might represent true findings and which may be statistical artifacts, resulting from the large number of comparisons inherent in whole genome scanning. Any or all of the linkages demonstrated may indeed be true findings, and it is noteworthy that a large number of loci are implicated in other mediated diseases, including insulin-dependent diabetes mellitus. Moreover, a number of these specific sub-chromosomal regions have also been implicated as being important in genome-wide searches in other immune-mediated diseases (31). These issues are discussed at length by a number of key investigators—notably Risch (32), Lander & Krugylak (33), and Schreiber (34), who have written influential reviews outlining guidelines for the interpretation and reporting of linkage results.

From linkage to gene?

In the 5 years that have passed since the initial results of genome-wide scanning in inflammatory bowel disease, clinicians and scientists have grown evermore impatient for the definitive identification for susceptibility genes. It is anticipated that this will allow early clinical application, and it is widely hoped that progress will lead to improvement in therapy for inflammatory bowel disease. Following convincing replication of a given linkage, the current challenge for investigators is to identify the genes within the region of linkage. However, each of the regions of linkage identified by affected sibling pair analysis in inflammatory bowel disease in the collaboration data-set is large and contains many known genes, together with as yet anonymous ESTs (exposed sequence tags). Thus the initial scientific challenge is to narrow the region of linkage. A number of strategies may be of use. World-wide collaborations have been set up so that data from affected sibling pair analysis might be shared, with the hope that the increased number of families might increase the power of study for fine-mapping. Unfortunately, however, the mega-analyses carried out to date have had limited success in precisely defining the region of linkage. Thus, whilst the published data from the International Inflammatory Bowel Disease Genetics Consortium successfully confirms linkage with chromosome 16 in Crohn disease, the data have not allowed narrowing of the region of linkage (17). Similarly, the combined Oxford–Pittsburgh collaboration has confirmed linkage between Chromosome 12 and ulcerative colitis, but has not substantially allowed fine mapping (19).

Indeed, there is a general consensus, supported by mathematical modeling, that non-parametric affected relative pair techniques, of the sort used to initially identify the regions of linkage, do not carry sufficient power in themselves to narrow the regions of linkage. This is largely the result of the limited number of meiotic events involved in the analyses of sibling pair families. Thus attention has switched to use of association studies, using either a case-control design or an intra-family 'transmission disequilibrium testing' (TDT) method. Compared with the non-parametric affected relative pair methods of linkage analysis, these association studies reflect the effect of many more meiotic events over generations, and therefore may have considerably increased power in refining the chromosomal location of susceptibility genes. Again, several comprehensive analyses of the benefits of this strategy have been performed, both in inflammatory bowel disease and in other complex diseases (34, 35).

Clinical considerations may also be of great benefit in refining areas of linkage and defining true susceptibility genes. Given the variability of clinical presentation seen in the inflammatory bowel diseases, which is likely to represent extensive genetic heterogeneity, a further advance may ensue from studying individuals of a particular disease behavior. It is apparent from the HLA studies of inflammatory bowel disease that the clinical variability of disease may well reflect genetic heterogeneity, and specific markers may predict progression in ulcerative colitis and the presence of complications of disease.

How then might phenotype be best defined? Phenotypic substratification of inflammatory bowel disease by extent, severity, or response to drug therapy is notoriously difficult. No consensus has yet been reached in subclassification. At present, age of onset of disease is undoubtedly the best-defined phenotype. In addition, there are strong epidemiological data which suggest that early onset inflammatory bowel disease has a stronger familial and genetic component than inflammatory bowel disease overall, a hypothesis which bears support by analogy with early onset breast cancer, colon cancer, and Alzheimer's disease. These epidemiological data are now supported by preliminary molecular genetic studies which indeed suggest that the linkages with chromosome 16 and chromosome 12 may be strongest in families in whom one member of the sibling pair has early onset inflammatory bowel disease (36, 37).

Positional candidate genes: a short cut to fine mapping?

In view of the difficulties inherent in fine mapping studies, alternative approaches to identifying susceptibility genes within regions of linkage have been explored. Access to

computer databases of genome sequence and data has become available to all scientists. Many potential candidate susceptibility genes have been mapped within the broad regions of linkage, and have consequently now been analyzed as potential susceptibility genes. Polymorphisms within these genes have been studied in inflammatory bowel disease in both linkage and case control studies. Of the potential candidates on chromosome 16, the genes encoding the interleukin 4 receptor, E cadherin and CD19 and 43 present themselves as the most compelling candidates. Strong negative data are available regarding the interleukin 4 receptor gene (38), but the other candidates remain under investigation.

On chromosome 12, a number of candidate genes lie within this broad region of linkage (39). These include the genes encoding the vitamin D receptor (40), a protein with a number of immuno-modulatory activities. Preliminary data have shown an association with Crohn disease but not ulcerative colitis for this protein. Recently, negative data have become available for the most attractive candidate gene within the chromosome 12 linkage interval, the adhesion molecule $\beta 7$ integrin, which has considerable specificity for the gastrointestinal tract (van Heel, Carey, Jewell, personal communication). Other molecules under investigation include the gene encoding the transcription factor STAT 6, and the metalloproteinase, MMP18.

Pharmacogenetics

The current literature reflects considerable optimism that studies of genetics will allow a more rational use of the medications currently available for the treatment of inflammatory bowel disease. There is hope that genetic studies will allow an understanding of the variability of efficacy of current therapeutics between individuals, and also a more detailed understanding of drug toxicities. Encouraging data have emerged regarding the identification of genetic determinants of efficacy and side effects of cortico-steroids, azathioprine, and antibodies to tumor necrosis factor (TNF). Gene expression studies, involving both intestinal biopsy and peripheral blood lymphocytes, suggest the genes encoding the multi-drug resistance protein (MDR) (41) and β subunit of the glucocorticoid receptor (42) as potential determinants of response to corticosteroids. Moreover, there are consistent data that polymorphism of the thiopurine methyl transferase enzyme, involved in the enzymatic activation of azathioprine, may well determine to a greater or lesser extent the efficacy or tolerability of this drug. Finally, there are preliminary data to suggest that polymorphisms of the gene encoding tumor necrosis factor alpha (TNFα) may indeed help predict the efficacy of this therapy in patients with Crohn disease.

Conclusions

Considerable progress has been made in the past 10 years in dissecting the genetic contribution to inflammatory bowel disease. Following compelling epidemiological data, molecular studies have provided strong evidence that Crohn disease and ulcerative colitis are related polygenic diseases. Non-parametric linkage analysis studies have been successful in identifying replicated regions of the linkage throughout the genome, and studies are now concentrated on fine mapping the genes within these regions. The early clinical application of these studies may be in determining the identity of those patients who will tolerate and benefit from the currently available immunosuppressants. However, there are great hopes for more direct and indirect benefits in the near future.

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