1. The aetiology of the chronic inflammatory bowel diseases, Crohn's disease and ulcerative colitis, is uncertain. Studies of specific environmental factors and immune dysfunction have provided little insight into disease pathogenesis.

2. Concordance rates in twin pairs and siblings provide strong evidence that genetic factors are important in disease pathogenesis. In Oxford, information was obtained from 433 adult patients with Crohn's disease. Compared with the prevalence in the general population, the relative risks in siblings of patients with Crohn's disease calculated from these data were respectively 36.5 for Crohn's disease, 16.6 for ulcerative colitis and 24.7 for inflammatory bowel disease.

3. Clinical patterns of disease were compared in members of over 250 multiply affected families with inflammatory bowel disease. A high degree of concordance for many characteristics was noted (disease type, extent, extra-intestinal manifestations). However, in 77 affected parent-child pairs, the median age of onset in the parents was significantly higher than in offspring \( P < 0.0001 \). These data reflect the results from other studies throughout the world, and are consistent with the phenomenon of genetic anticipation.

4. A detailed study investigating the contribution of the major histocompatibility complex was undertaken. Eighty-three affected sibling pairs were involved in a linkage analysis study; 348 patients with inflammatory bowel disease and 472 controls were involved in a detailed allelic association study. These data provide evidence that the major histocompatibility complex is an important determinant in ulcerative colitis, but not in Crohn's disease.

5. Cytokine genes are important candidate genes in inflammatory bowel disease. Allelic association study was performed to investigate the contribution of the gene encoding the interleukin-1 receptor antagonist and tumour necrosis factor-\( \alpha \). These data do not suggest that these genes encode important determinants of disease susceptibility in inflammatory bowel disease.

6. A two-stage genome-wide search for susceptibility loci in inflammatory bowel disease was performed involving 186 affected sibling pairs. The data provide strong evidence for the model of Crohn's disease and ulcerative colitis as related polygenic disorders. Loci on chromosomes 3, 7 and 12 were linked to inflammatory bowel disease overall, whereas loci on chromosomes 2 and 6 were linked only in ulcerative colitis. Linkage with chromosome 16 was noted in Crohn's disease only. Fine mapping of these susceptibility loci is in progress, and may lead to gene identification with attendant clinical benefits.

INTRODUCTION

Crohn's disease and ulcerative colitis, the chronic inflammatory bowel diseases, are common causes of gastrointestinal illness in young people in the Western world. Crohn's disease may affect any part of the gastrointestinal tract, and is characterized by the presence of discontinuous areas of transmural inflammation, whereas ulcerative colitis typically is confined to the colonic mucosa. However, in perhaps 10% of cases of inflammatory bowel disease affecting the colon, it is impossible to distinguish between Crohn's disease and ulcerative colitis. Both diseases may have similar extra-intestinal manifestations, affecting the liver, eyes, joints or skin. Carcinoma of the colon may complicate extensive ulcerative colitis or Crohn's disease of the colon.

The aetiology of these disorders, and even the precise nature of the relationship between Crohn's disease and ulcerative colitis, remains uncertain. Studies of specific environmental pathogens or immunological abnormalities have failed to provide meaningful insight into the primary pathophysiology involved in disease susceptibility or disease progression. It is only recently that the importance of genetic susceptibility in the pathogenesis of inflammatory
bowel disease has been subject to re-evaluation. Here, recent progress made in understanding the genetic basis of inflammatory bowel disease is reviewed, with particular reference to work carried out in Oxford. These studies have provided strong evidence to suggest that the inflammatory bowel disorders are related diseases of polygenic inheritance. The data suggest that the variability of clinical presentation of inflammatory bowel disease may reflect extensive genetic heterogeneity, involving not only different genes, but allelic variation within a single gene.

If sufficient clinical and financial resources are available, significant progress in understanding the pathophysiology of the inflammatory bowel diseases seems likely. This understanding may provide the basis for direct clinical and therapeutic applications.

FAMILIAL PREVALENCE OF INFLAMMATORY BOWEL DISEASE

Many studies have demonstrated an increased prevalence of inflammatory bowel disease among relatives of patients with Crohn’s disease and ulcerative colitis [1,2]. Consistent trends are noticeable. First-degree relatives are at greatest risk, particularly siblings, but more distant relatives also display an increased disease prevalence. A positive family history is more common in relatives of patients with Crohn’s disease than ulcerative colitis.

Estimates of the prevalence of a positive family history vary considerably from study to study. In particular, many of the data implying particularly high disease prevalence rates in first-degree relatives may be criticized for using selected patient groups. Ethnicity (notably Ashkenazi Jews) [3] and young age of disease onset appear to be associated with the highest prevalence of familial disease.

In Oxford [4], information was obtained from 433 adult patients with Crohn’s disease using a postal questionnaire, review of case notes and personal interviews. More than 95% of patients were resident locally in Oxfordshire, and referrals from outside the region were deliberately excluded. All patients were North European Caucasians, and less than 5% of the patients were of Jewish ancestry. In 78 families (18%), at least one first- or second-degree relative also had inflammatory bowel disease. Three relatives were affected in 14 families, and four in two families. Both Crohn’s disease and ulcerative colitis occurred in the multiply affected pedigrees. First-degree relatives were affected in 50 families (11.5%). Siblings were most commonly affected. Crohn’s disease was more common than ulcerative colitis in the affected siblings. There was no increased risk of inflammatory bowel disease in spouses or adopted family members.

Assuming population prevalences of 70/100000 for Crohn’s disease and 100/100000 for ulcerative colitis, the relative risks in siblings of patients with Crohn’s disease calculated from these data were respectively 36.5 for Crohn’s disease, 16.6 for ulcerative colitis and 24.7 for inflammatory bowel disease.

These data are consistent with results of previous studies of familial inflammatory bowel disease in the U.K. Probert et al. [5] investigated the prevalence and family risk of ulcerative colitis and Crohn’s disease among Europeans and South Asians in Leicestershire. From their data, these authors calculated values of relative risk of Crohn’s disease in siblings, parents and offspring of patients with Crohn’s disease as 34.7, 17.1 and 29.1 respectively.

These data complement the results of the Swedish twin study [6] which strongly implicated genetic susceptibility in the pathogenesis of Crohn’s disease and ulcerative colitis. In that study, the coefficient of heritability derived from twin concordance rates was greater in Crohn’s disease than in many common disorders (notably schizophrenia and hypertension). The magnitude of relative risks in siblings has further implications. Data reported from the Oxford study, and from previous studies, are not in keeping with either Crohn’s disease or ulcerative colitis having a simple monogenic pattern of inheritance. The model of disease inheritance which appears most pertinent to these common disorders is that of polygenic inheritance. Moreover, given the clinical variability of presentation in inflammatory bowel disease, the most appropriate model may incorporate not only polygenic inheritance but also genetic heterogeneity. The term ‘inflammatory bowel disease’ may truly represent a group of related polygenic disorders sharing some but not all susceptibility loci. Clinical presentation may depend on the interaction between different genes, and also on allelic variation in the individual genes.

Risch [7] has demonstrated that in such complex disorders, the magnitude of familial clustering (expressed as the relative risk to the sibling of a proband, a(s)) provides a direct reflection of the ease of mapping susceptibility loci. The values derived for Crohn’s disease in the Oxford study may be compared with values derived in insulin-dependent diabetes (a(s) = 15), a disease in which genome-wide screening has been applied successfully.

CLINICAL PATTERNS OF INFLAMMATORY BOWEL DISEASE

In contrast to rare monogenic disorders, dissection of the genetics of complex traits necessitates the identification of very large numbers of multiply affected families. In inflammatory bowel disease, many groups have set up detailed programmes to identify multiply affected families. The clinical data collected from such studies may prove of considerable value in identifying the extent of disease heterogeneity and the influence of genetic and environmental factors on disease behaviour. Recent data from studies in France [8] and the U.S.A. [9] have demonstrated marked concordance in members of multiply affected families with Crohn’s disease for disease extent and behaviour.

In Oxford, a repository of clinical material (DNA, plasma and frozen lymphocytes) from over 250 multiply affected families has been established [10].
Wherever possible, full details of clinical history were obtained and stored on a computer database. Fifty-four families in whom one parent and at least one child were affected (a total of 77 parent–child pairs were available) and 155 families in whom at least two affected siblings were present (a total of 190 affected sibling pairs) were involved in these clinical studies. All families involved were North European Caucasians resident in the U.K. Less than 5% of families claimed Jewish ancestry.

In affected parent–child pairs, parent and child were concordant for disease type in 58 of 77 pairs (75.3%), for extent in 63.6%, extra-intestinal manifestations in 70.1% and smoking history in 85%. The median age at diagnosis in parents was significantly higher than in offspring ($P < 0.0001$). In 40 pairs, the parent was at least 10 years older than the child at onset of disease.

Siblings were concordant for disease type in 81.6% of the affected sibling pairs, extent in 76.0%, extra-intestinal manifestations in 83.8% and smoking history in 81.3%. In contrast to the parent–child pairs, 68.1% of siblings were diagnosed within 10 years of one another. The median age of onset was 24 years.

These data complement previous studies, demonstrating consistent clinical patterns in multiply affected families with inflammatory bowel disease. However, of particular note in the present study are the differences in the age of onset between affected parents and their children. Similar data have now been presented from many centres concerning parent–child pairs in the U.S.A. and Europe. In a study from the Johns Hopkins Hospital [9] the children were not only significantly younger at age of onset of disease, but generally also suffered more extensive disease.

The explanation for this observation is uncertain. A simple cohort effect due to simultaneous exposure to environmental pathogen was not apparent in the present study. Simple reporting bias also appeared unlikely, given the marked discrepancy in age of onset between parent and child. Polito et al. [9] have suggested that the observed age differences may represent genetic anticipation. This phenomenon, the tendency for successive generations to develop disease of increasing severity and earlier onset, has been observed in monogenic disorders, particularly neurodegenerative diseases. The molecular basis for this effect involves the progressive amplification of unstable triplet repeats of DNA [11]. Whether this explanation is pertinent to complex disorders such as inflammatory bowel disease, or whether the observations reflect ascertainment bias, or some other variable [12], is uncertain; these data are subject to re-evaluation.

**CONTRIBUTION OF GENES OF THE MAJOR HISTOCOMPATIBILITY COMPLEX**

Data concerning the importance of the genes of the major histocompatibility complex in Crohn’s disease and ulcerative colitis have, until recently, remained confusing and inconsistent, except for those small subgroups of patients with concomitant ankylosing spondylitis (associated with HLA B27) or primary sclerosing cholangitis (associated with HLA DR3 DQ2 or HLA DR2) [2]. Allelic associations have been reported between HLA DR2 (HLA DRB1*1502) and ulcerative colitis in Japanese [13] and Jewish [14] patients. However, this allele is rare in non-Jewish European patients. A further controversy is the relationship between HLA genotype and disease progression.

A detailed study investigating the contribution of the HLA DRB1 and DQB genes to disease susceptibility and phenotype in inflammatory bowel disease was undertaken in Oxford, using the strategies of non-parametric linkage analysis (affected sibling pair method) and association studies. In total, 83 affected sibling pairs were involved in the linkage analysis study (42 pairs in which both siblings had Crohn’s disease, 29 in which both had ulcerative colitis, and 12 mixed disease pairs). The allelic association study included 175 patients with ulcerative colitis, 173 patients with Crohn’s disease and 472 control subjects. HLA DRB1 and DQB1 genotyping was performed by polymerase chain reaction using sequence-specific primers [15].

The results are consistent with genetic heterogeneity both between Crohn’s disease and ulcerative colitis, and within ulcerative colitis. The sharing of alleles among affected sibling pairs with ulcerative colitis provides evidence for linkage with the DRB1 locus ($P = 0.017, \chi^2 = 5.32$). Of 29 affected sibling pairs studied, only one pair shared no DRB1 DQB haplotypes; 15 shared both haplotypes. In contrast, no linkage was reported in Crohn’s disease ($P = 0.30, \chi^2 = 0.16$) or for inflammatory bowel disease overall ($P = 0.16, \chi^2 = 2.28$).

In the allelic association study, the rare DRB1 103 (8.3% versus 3.2% in controls) and DRB1 12 (8.6% versus 2.1% in controls) alleles were associated with ulcerative colitis overall ($P = 0.0074, \chi^2 = 7.22$, odds ratio (OR) = 2.9 (95% confidence intervals 1.3–6.4) and $P = 0.0056, \chi^2 = 12.63, \text{OR} = 4.33$ (1.8–11) respectively). No association with alleles representing DR2 was noted.

No overall association was seen in Crohn’s disease. In ulcerative colitis, the frequency of the DR3 DQ2 haplotype was reduced in females [9.8% versus 26.3% in controls; $P = 0.037, \chi^2 = 8.39, \text{OR} = 0.34$ (0.51–0.71)], particularly in those with distal disease [2.3%; $P = 0.001$ versus controls, $\chi^2 = 11.35, \text{OR} = 0.07$ (0.00–0.39)].

In both males and females, the DR3 DQ2 haplotype was predictive of extensive ulcerative colitis [32.9% versus 10.7% in distal disease; $P < 0.01, \chi^2 = 10.94, \text{OR} = 4.09$ (1.70–10.6)], but not of need for surgery ($P = 0.93, \chi^2 = 0.01$).

These data have considerable implications. Genes of the major histocompatibility complex influence not only disease susceptibility, but also disease progression in ulcerative colitis. In Crohn’s disease, the important
susceptibility genes do not appear to exist within the major histocompatibility complex.

The allelic associations reported in these patients are subject to evaluation in other populations. In Oxford, an independent study has confirmed that HLA DRB1*103 may not only predict severe disease requiring surgery, but is particularly associated with the presence of extra-intestinal manifestations (iritis, mouth ulcers, arthropathy) [16].

OTHER CANDIDATE GENES

Other genes involved in the regulation of the immune response or maintenance of mucosal integrity represent candidate genes in inflammatory bowel disease. Of particular present interest are the genes encoding the naturally occurring antagonist to interleukin-1, interleukin-1 receptor antagonist, and the gene encoding the pro-inflammatory cytokine tumour necrosis factor-α. Both genes contain polymorphisms of potential functional significance [17,18] in determining gene transcription and protein concentrations. However, data from Oxford [19] involving 129 patients with ulcerative colitis, 120 patients with Crohn's disease and 89 healthy controls do not suggest that these genes are important determinants of overall disease susceptibility. Modest associations only (Table 1) were demonstrated, in contrast to reported results from elsewhere in the U.K. [20] and North America [21]. Disease heterogeneity and ethnic differences may be pertinent. These and other candidate genes remain under evaluation. Preliminary data from Oxford suggest that the genes encoding interleukin-2 [22] and interleukin-10 [23], both implicated in animal models, are not strong overall determinants of disease susceptibility either in Crohn's disease or ulcerative colitis. However, these studies require replication in independent data-sets before final conclusions are possible.

**Table I** Cytokine gene polymorphisms in inflammatory bowel disease: allelic frequencies (%) for the variable number of tandem repeat polymorphisms in intron 1 of the interleukin-1 receptor antagonist gene

<table>
<thead>
<tr>
<th>Allele</th>
<th>Allele 2</th>
<th>Allele 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcerative colitis (113 patients)</td>
<td>72.6</td>
<td>24.3</td>
</tr>
<tr>
<td>Crohn's disease (115 patients)</td>
<td>72.6</td>
<td>24.7</td>
</tr>
<tr>
<td>Controls (89 subjects)</td>
<td>76.9</td>
<td>20.8</td>
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**GENOME-WIDE SCANNING IN INFLAMMATORY BOWEL DISEASE: EVIDENCE FOR NOVEL LINKAGES**

Systematic screening of the entire human genome now provides a practicable strategy for the identification of susceptibility loci in complex polygenic disorders. Since the initial application of this methodology in insulin-dependent diabetes [24,25], genome-wide scanning has become the focus of intensive scientific interest. Increased automation has allowed rapid large-scale genotyping projects to be performed in many complex diseases. However, data from recent studies do serve to highlight the need for rigorous statistical design. Studies must be of sufficient power to detect genes with relatively small effect without incurring a large number of false-positive linkages. The design of such studies is the subject of particular controversy in the genetics community, but consensus opinion is that in complex diseases, these considerations require staged screening of large numbers of pedigrees.

Hugot et al. [26] reported the first such study in inflammatory bowel disease. This collaborative study, involving a total of 78 multiply affected families with Crohn's disease from throughout Europe, identified a single susceptibility locus on chromosome 16, which contributed a locus-specific relative risk to siblings of 1.3. It is noteworthy that this linkage has been reproduced relatively easily in independent data-sets in Europe [27] and the U.S.A. [28]; indeed, this ease of replication contrasts with data in other disorders.

However, as the authors themselves conceded [26], this locus explained at most 10% of genetic susceptibility associated with Crohn's disease. The failure of this initial study to identify other loci may reflect
either the limited power of the study to pick up relatively weak genetic determinants, or extensive heterogeneity within Crohn's disease itself.

In Oxford, a two-stage genome-wide search was performed involving a total of 186 affected sibling pairs with inflammatory bowel disease [29]. In 81 sibling pairs both had Crohn's disease, in 64 pairs both had ulcerative colitis, and in the remaining 41 pairs one sibling had Crohn's disease and the other ulcerative colitis. In the first stage of investigation, 89 affected sibling pairs were genotyped at 260 microsatellite markers spanning the 22 autosomes, using fluorescence labelled primers for polymerase chain reaction, and semi-automated DNA fragment sizing technology. In the second stage, a further 97 affected sibling pairs were genotyped at 16 microsatellite markers to investigate linkages suggested in the first data set.

The first stage provided preliminary evidence for linkage between susceptibility to inflammatory bowel disease and 12 markers in five distinct regions, on chromosomes 2, 3, 7, 12 and 16 (P < 0.001 for an individual marker, or adjacent markers each with P < 0.01). The data from the second set of families clearly replicated the results from stage 1 for the clustered markers on chromosomes 3, 7 and 12 (Table 2).

Combining data from the first and second stages provided striking evidence for linkage between susceptibility to inflammatory bowel disease and regions on chromosomes 3, 7 and 12. Linkage was noted with four adjacent markers on chromosome 12 [D12S83 (P = 2.66 × 10-7, lod score 5.47), D12S92 (P = 2.14 × 10-9, lod score 4.59), D12S368 (P = 5.03 × 10-5, lod score 3.29) and D12S43 (P = 0.0011, lod score 2.03)], three adjacent markers on chromosome 7 [D7S1519 (P = 1.33 × 10-4, lod score 2.89), D7S669 (P = 8.20 × 10-8, lod score 3.08) and D7S524 (P = 8.45 × 10-5, lod score 2.14)], and two adjacent markers on chromosome 3 [D3S1573 (P = 2.1 × 10-4, lod score 2.69) and D3S1076 (P = 0.0027, lod score 1.69)].

Multipoint analysis and fine mapping studies have further narrowed the region of linkage on chromosome 12 [30]. Furthermore, individual markers on chromosomes 2, 6 and 16 were linked with susceptibility to ulcerative colitis (P = 0.0071 and P = 0.024) and Crohn's disease (P = 0.004) respectively.

IMPLICATIONS

These data provide perhaps the strongest evidence to date that Crohn's disease and ulcerative colitis truly are related polygenic disorders sharing some but not all susceptibility loci. The data provide clear direction for further studies. Replication in independent data sets of these linkages is a priority; preliminary data from Germany and the U.S.A. provide confirmation for the linkage with chromosome 12. Precision mapping of the linked regions, ideally to within 1 centi-Morgan, is the next priority; this is likely to require very considerable clinical and financial resources, such that one centre alone is unlikely to be able to carry out this work. International collaboration may accelerate these activities. Once the precision mapping studies have been completed, physical mapping and gene identification become a realistic possibility. Even at this stage, confirmation of the role of any putative gene is likely to require extensive mutation analysis, and animal studies.

The regions on chromosomes 3, 7, 12 and 16 are known to contain a number of potential candidate genes; it is possible that immediate study of these genes may provide a short-cut to the strategy outlined above. A number of these positional candidates are particularly noteworthy: on chromosome 3, the gene encoding GNAI2 (an inhibitory G-protein subunit, implicated in animal work); on chromosome 7, the gene encoding MUC3 (the protein chain of one of the commonly expressed intestinal mucin glycoproteins), hepatocyte growth factor and epidermal growth factor receptor; and on chromosome 16 the gene encoding E-cadherin and the receptor for interleukin-4. Mutation analysis and sequencing of these candidates is already underway.

In the last few years, considerable progress has been made in dissecting the genetic basis of the inflammatory bowel diseases. There is now a very real hope that these studies will lead to an understanding of the basic pathophysiology of these complex disorders with clinical and therapeutic benefits.

REFERENCES