# Genetics of inflammatory bowel disease — an update

D. A. van Heel, D. P. Jewell

Wellcome Trust Centre for Human Genetics and Gastroenterology Unit, University of Oxford, U.K.

## Introduction

The pathogenesis of ulcerative colitis (UC) and Crohn's disease (CD) is still unknown, but epidemiological studies show clear evidence of genetic susceptibility. Recently this evidence has been supplemented by molecular genetic data from candidate gene studies and genome wide linkage scans. Four sub-chromosomal regions likely to contain susceptibility genes have been identified in linkage studies and replicated by independent investigators. These efforts should allow better understanding of disease pathogenesis and may lead to new targeted therapies.

## Evidence for a genetic susceptibility to IBD

Genes are unequivocally shown to be involved in disease susceptibility when there is a consistent Mendelian segregation pattern or a chromosomal abnormality. Although these features have not been found in IBD, genetic susceptibility has been strongly suggested by ethnic differences in disease prevalence, familial clustering of disease, twin concordance rates, and, most recently, data from molecular studies in man and in animal models.

Certain ethnic groups have a consistently higher risk of developing IBD, these include Ashkenazi Jews who have a 2-4× increased risk of IBD (especially CD) compared to the 150-200 per 100,000 observed in Northern Europe (1-10). The highest prevalence of family history in IBD is found in early onset cases. A 29% family history was reported for IBD in UC patients and 35% in CD patients when the disease was diagnosed before 21 years old (11). Familial clustering can be measured by the ratio of risk to siblings compared with the general population risk, estimated at 15-35 for Crohn's disease and 7-12 for ulcerative colitis (12-15). Concordance for CD location and type within families has also been shown by several centres.

Twin studies have been widely used in human genetics to study the relative contributions of genes and environment. Di- and monozygotic twins share half and nearly all their genes respectively, but also share to a large extent the same environment – which is thus controlled for. Combined data from the British, Danish and Swedish twin studies give overall UC mono- and dizygotic concordance rates of 13% and 2% respectively compared with 33% and 4% for CD. The higher degree of heritability for CD than UC is consistent with the family study data.

Many studies have been carried out of models of inheritance of inflammatory bowel disease. Complex segregation analysis has been used to model inheritance of IBD, and has found evidence for a major dominant or additive disease gene in 10% of UC families (16,17). Studies in CD have suggested that between 7 and 30% of patients may be homozygous for a recessive disease gene (although this could also be explained by a multifactorial model) (17,18). Current theories model CD and UC as a group of oligogenic diseases sharing some susceptibility genes but differing at others. Multiple disease alleles (themselves perhaps of weak effect) may act together, and once a threshold is reached IBD may occur if environmental risk factors (e.g. smoking, gut microflora) are present.

## Linkage studies and IBD susceptibility loci

In linkage analysis, multiply affected families are genotyped for polymorphic markers. Markers are sought where the degree of allele sharing between affected individuals exceeds that expected by chance (for sibling pairs > 50%). A marker close to a disease gene is less likely to be changed by meiotic recombination than one far away, and if the same allele of a disease gene is involved in both affected siblings then the siblings will share the same allele at the nearby marker. A sub-chromosomal region can then be identified which is genetically linked (i.e. co-inherited) with an as yet unknown disease susceptibility gene or genes. In order to adequately cover the whole genome a set of about 300 markers are needed (10cM intervals). This leads to a problem of multiple testing, difficult to resolve, but which either needs stringent thresholds (e.g. "significant linkage" of $P < 2 \times 10^{-5}$) or replication in other datasets. Newer methods of linkage analysis do not involve specification of a genetic model (non parametric) and use data from multiple markers simultaneously (multipoint).

Genome scans have now been undertaken by many groups working in IBD genetics. A large number of loci have been identified, which will undoubtedly contain false positives (see above). These data have been added

Corresponding author : Jewell, D. P., Gastroenterology Unit, Radcliffe Infirmary, Gibson Building, 2nd Floor, Woodstock Road, Oxford OX2 6HE, U.K.

to by other groups studying the most promising linkage regions only. Recently an international IBD Genetics consortium has analysed data in a large number of families from multiple groups, in order to increase the power of the studies. Replicated loci have been found on chromosome 6, 12, 14 and 16. In the summary of these published studies below, note that there are differences in phenotype, analysis method and study size between the papers which complicate direct comparison.

| Chr | Study | Families | Multipoint p |
|---|---|---|---|
| 6 (MHC) | Hampe *et al.* 1999 | 268 | p = 0.003 |
| 6 (MHC) | Rioux *et al.* 2000 | 158 | p = 0.0005 |
| 6 (MHC) | Ma *et al.* 1999 | 46 | p = 0.01 |
| 6 (MHC) | Oxford (ASHG 2000) | 236 | p = 0.0002 |
| 12q13-15 | Satsangi *et al.* 1996 | 160 | p = 0.0000003 * |
| 12q13-15 | Duerr *et al.* 1998 | 122 | p = 0.0002 |
| 12q13-15 | Hampe *et al.* 1999 | 268 | p = 0.002 |
| 12q13-15 | Yang *et al.* 1999 | 46 | p = 0.0004 |
| 14q11-12 | Duerr *et al.* 2000 | 62 | p = 0.00002 |
| 14q11-12 | Ma *et al.* 1999 | 46 | p = 0.0002 |
| 16 centro | Hugot *et al.* 1996 | 78 | p = 0.00002 |
| 16 centro | Ohmen *et al.* 1996 | 48 | p = 0.0004 |
| 16 centro | Cavanaugh *et al.* 1998 | 54 | p = 0.000002 |
| 16 centro | Cho *et al.* 1998 | 174 | p = 0.003 |
| 16 centro | Hampe *et al.* 1999 | 268 | p = 0.003 |
| 16 centro | Annese *et al.* 1999 | 58 | p = 0.003 |

* singlepoint, recessive model

In summary, strong evidence for linkage exists for chromosomes 6, 12 and 16. In addition chromosome 14 has been demonstrated by two groups. Not all studies have been able to replicate previously identified susceptibility loci. This is a common finding in complex disease genetics and can be due to lack of statistical power, ethnic differences between populations, variability in phenotype studied amongst others.

## Methods underlying candidate gene association studies

Association studies can identify disease alleles with a weak effect, which can be missed with linkage studies. Association studies may identify either the disease allele itself, or one in linkage disequilibrium with it. Note that linkage disequilibrium operates over small distances (max 1Mb although highly variable) compared to the larger 10-20Mb regions defined by linkage studies.

Case-control association studies compare allele frequencies in affected patients to a set of matched controls. False positive associations may be seen due to mismatching of controls, possibly due to population stratification/ admixture. The transmission disequilibrium test (TDT) avoids this problem by using the parental alleles as internal controls. Future studies in IBD are likely to use the TDT more widely as families are collected, due to its power and robust nature. A further problem of association studies is that there may well be no association to find, and so each test performed carries an independent risk of a false positive result (19). The best test of an association is replication by different investigators in a separate patient group.

### Positional candidate genes

Identification of genes within a region of linkage (positional candidates) has been made simpler by the publication of a physical map of nearly half of all predicted human disease genes (http://www.ncbi.nlm.nih.gov/genemap99). Using this information and the increasing quantities of genomic sequence from the Human Genome Project, it is possible to easily identify promising positional candidates. Candidate genes for IBD on chromosome 6 include HLA class I and II, TNFα, MICA and MICB. ITGB7, STAT6, IFNγ NRAMP2, VDR are found on chromosome 12q13-15. The centromeric region of chromosome 16 contains ITGAX, CD19 and IL4R.

Interferon-gamma (IFNγ, chr. 12q14) is a key Th-1 cytokine, and abnormalities of this pathway are well described in animal models and in humans with inflammatory bowel disease. An intragenic microsatellite marker was typed in 506 simplex families (i.e. patients and parents) and 133 affected relative pairs. The transmission disequilibrium test was negative for CD, UC and IBD overall (20). The authors propose that abnormalities related to IFNγ observed in IBD are likely to be a secondary phenomenon.

Vitamin D has multiple immune actions, including the suppression of lymphocyte proliferation and inhibition of cytokine production. The Taq1 t allele and tt genotype of the vitamin D receptor gene (chr. 12q13) were significantly associated with Crohn's disease (21). This genotype has been reported to be of functional importance in several infectious diseases.

NRAMP2 (Chr 12q13) is involved in intestinal iron transport and has homology to NRAMP1 (involved in TB susceptibility). Three restriction fragment length polymorphisms were assessed, and the gene excluded as a candidate by linkage and TDT analysis. No novel mutations were found by direct sequencing of the gene in 33 CD patients.

Positional candidates on chromosome 16 include the CD19 gene and CD43 (sialophorin) gene, involved in B lymphocyte function and ICAM1 interactions respectively. Direct sequencing of the coding sequence of these genes, including intron-exon junctions, was carried out in 10 CD patients and 2 healthy controls. Two single nucleotide polymorphisms were identified in each gene, three of which were silent mutations and one a single base amino acid missense mutation in CD19 exon 3. The TDT was negative for the CD19 missense mutation in 78 CD families. Although rare alleles may have been missed, the CD19 and CD43 genes did not appear to be involved in the genetic predisposition to CD in these families (22).

### The MHC and IBD

Amongst the first candidate genes to be studied in IBD were those of the HLA system, involved in antigen processing and presentation. HLA class II molecules present partially digested antigen to the T cell receptor, and play a central role in the immune response. Conflicting results in HLA association studies are common (see above for reasons). The most consistent results have been seen in Japan, where the most genetically homogenous population is found. The strongest association are of DRB1*1502 in UC (esp. Japanese) and DRB1*03 as a resistance allele in Crohn's disease.

Many other genes have been studied in IBD on the basis of potential function alone. The interleukin-1 receptor antagonist (Il-1 RA, chr. 2q) is a potent anti-inflammatory protein, implicated in intestinal inflammation from animal studies. An initial case-control study in UC and CD found an association in UC patients with allele 2 of an intragenic VNTR (35% vs 24% controls) (23). In Oxford no significant differences between CD, UC and controls were initially seen (24). Analysis of subgroups in UC showed allele 2 to be increased in ANCA positive UC patients, and more frequent in extensive disease than distal colitis (25). Interleukin-1 beta and receptor antagonist genes were not associated with UC overall in a Dutch study, but allele 2 of Il-1 RA was increased in extensive disease (26).

Intracellular adhesion molecule 1 (ICAM-1, chr. 19p13) is involved in neutrophil adhesion and two exonic polymorphisms have been tested in UC and CD patients. No association was found for CD or UC overall. After stratification by ANCA status, ANCA positive CD and ANCA negative UC had an increased frequency of allele R241 (27). Codon 241 is in a functionally important domain of ICAM-1, and this interesting association awaits replication by other groups.

The gene encoding tumor necrosis factor alpha (TNFα, chr. 6p21) has been studied because of well described alterations of TNF production in IBD, and because of the efficacy of anti-TNF monoclonal antibodies in CD (28). Many TNFα polymorphisms have been described, of which the –308 and –238 promoter mutations have been suggested to be functional (29). As for the IL-1RA polymorphisms, data from several published studies and analysis of subgroups have been discordant (23,24,30). Investigators in Los Angeles found a TNFα microsatellite haplotype (a2b1c2d4e1) in 24% of CD patients versus 7% of controls, although a French group did not (31,32). This haplotype is in linkage disequilibrium with HLA-DR and DQ alleles, and dissection of the pathological mutation (versus polymorphism) in this region of chromosome 6 will be needed.

Chemokines are released by intestinal epithelium, playing a major role in recruitment and activation of inflammatory cells. The chemokine receptor CCR-5 (chr. 3p21) has a 32bp deletion found to confer resistance to HIV, and is close to a linked marker D3S1573 (33). 44% of CD patients and 31% of UC patients versus 13% of controls possessed this functional deletion (34).

Abnormalities of intestinal mucin expression have been observed in IBD patients and the cotton-top tamarin model of colitis. The MUC3 gene (chr. 7q22) lies close to a microsatellite D7S669, linked to IBD with a lod score of 3.08 in the Oxford genome scan (33). Polymorphisms of variable number of tandem repeats (VNTRs) within the MUC3 gene in 75 Japanese patients with ulcerative colitis and 168 Japanese controls were analysed. When the frequency of patients carrying 1 or 2 rare VNTR alleles was compared with that of controls, a significant increase was found in the Japanese patients (odds ratio 2.72). Similar results were found in Caucasians. Rare alleles of MUC3 may confer predisposition to UC, although these were found in only 22% of UC patients studied (35).

## Conclusions

Inflammatory bowel disease genetics has advanced rapidly in the past five years. Earlier data were limited to epidemiological and serological HLA association studies. Molecular biological techniques have progressed through genome wide linkage studies to widespread replication of regions of linkage and the analysis of positional candidate genes. In particular, IBD is fortunate amongst complex genetic disease in having replicated non-HLA susceptibility.

The relatively small role HLA genotypes have in IBD susceptibility is becoming clearer. HLA DRB1*0103 and DR2 (DRB1*1502) are involved in UC susceptibility in Caucasian and Japanese populations respectively, whilst DRB1*03 and DR4 appear to be resistance alleles for CD and UC. Studies in arthropathy and of different clinical types of IBD suggest that HLA may have a greater role in modifying IBD phenotype, than on overall disease susceptibility.

Replicated regions of linkage span over 10-20 million base pairs, regions which could each contain several hundreds of genes. Identification of susceptibility genes is thus a formidable prospect, and strategies proposed include analysis of subgroups, fine mapping, meta-analysis, and a systematic analysis of all candidates. Fine mapping of these loci using a dense set of microsatellite markers is being attempted, with some promising initial results (36). In contrast to simple mendelian traits where the gene always lies within the maximal region of linkage, in complex disease the gene may well lie outside the maximal region (37). In support of this argument the positive studies for chromosome 12 include three peaks of linkage each 10cM apart. It is also possible that more than one susceptibility gene may reside within the region (38-40). The power of linkage studies to narrow a region is limited, indeed over 700 sib

pairs may be needed to narrow down to 1cM a locus causing a twofold increased risk (41).

New developments in association studies, such as the TDT, provide more statistically robust methods of testing than the traditional case-control study. Two studies have suggested linkage disequilibrium for a microsatellite marker D12S83 on chromosome 12 (36,38). However recent statistical modelling suggests that useful levels of linkage disequilibrium in an outbred population may extend over very small regions (3 kB on average, although with wide regional variation) (42). Microsatellite markers may well be unable to provide sufficient resolution, being too widely spaced, and other markers such as single nucleotide polymorphisms (SNP's) may hold more promise. An alternative strategy is to identify all the potential genes within the large regions of linkage and apply methods for detection of novel mutations. The more likely candidates may be identified on the basis of known or predicted function and tissue specific expression. Systematic analysis of positional candidate genes, starting with the most plausible biological candidates, is likely to progress in parallel with attempts to narrow the regions of linkage.

Identification of a susceptibility gene and characterisation of the functional significance of mutations has the potential to unravel the molecular pathogenesis of IBD. This might allow new therapeutic measures to be developed and allow investigation of potential environmental trigger factors. Clinical practice may include stratification of patients by genotype and potential response to drug therapy. For example, susceptibility to the myelosuppressive effects of azathioprine is now partly understood at the molecular level (43). Genetic counselling both of individual patient and their relatives may eventually become possible. Tremendous progress has been made in IBD genetics over the past five years, and there is great optimism that further advances will follow rapidly.

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