Progress in searching for susceptibility gene for inflammatory bowel disease by positional cloning

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INTRODUCTION

Inflammatory bowel disease (IBD) is composed of two clinical subtypes: Crohn disease (CD) and ulcerative colitis (UC). Its general prevalence is about 1.0-2.0 % in Western countries. It is predominantly regarded as a multifactorial disorder involving environmental factors and polygenic defects. The view was confirmed by a lot of evidences from clinical attributions and animal models, especially from epidemiological investigations. So the etiological study of IBD has been focused on searching for susceptibility genes by positional cloning, which consists of two steps: linkage analysis and association analysis. Linkage analysis has been an important method of searching for susceptibility genes to polygenic diseases as well as single-gene disorders. IBD, as a polygenic disease, has been widely investigated by linkage analysis for susceptibility gene since 1996. The paper reviewed 38 articles, which covered almost all original researches in relation to IBD and linkage analysis. So far, several loci, such as 16q, 12q, 6p, and 3p, have been identified by the studies. The most striking is 16q12 (IBD1), which linked only with CD not UC in the majority of studies. Association analysis, as one essential step for positional cloning, is usually carried out by genotyping candidate genes selected by means of linkage analysis or other methods, for figuring out the frequencies of alleles and comparing the frequencies between IBD group and healthy control group to identify the specific allele. It has been established that IBD is implicated in immune disorder. So the studies were centered on the genes of NOD2/CARD15, HLA-II, cytokine, cytokine receptor and adhesion molecule. This article also comprehensively analyzed 18 original researches of HLA gene polymorphism in IBD. We found extensive discrepancy among the conclusions and a novel hypothesis was put forward to explain the discordance. Most studies published recently on association between IBD and cytokine gene polymorphism were reviewed.

Zheng CQ, Hu GZ, Zeng ZS, Lin LJ, Gu GG. Progress in searching for susceptibility gene for inflammatory bowel disease by positional cloning. World J Gastroenterol 2003; 9(8): 1646-1656 http://www.wjgnet.com/1007-9327/9/1646.asp

B. There have been a number of hypotheses about the pathogenesis of IBD, but neither environmental factors, such as habits of diet and behavior, infection of microorganisms and contact of chemical or physical pathogenic agents, nor single-gene genetic disorder alone can fully explain its complex phenotypes. Therefore, it is thought to be a multifactorial disease. The view was supported by a larger amount of evidences from clinical attributions and animal models, especially epidemiologic investigations and linkage analyses.

Persuasive evidences of genetic contribution to IBD

A. The first-degree relatives of affected individuals show about 20-50-fold increased risk of developing the disease compared with the general population for CD, and 10-20-fold increased risks for UC. Moreover, the affected siblings frequently present at similar ages and concordance rates reach up to 80 % for disease site, behavior and presence of extraintestinal manifestation. B. Twin studies have shown that the concordance rate of CD is about 20-44 % for monozygotic twins, and 3.8-6.5 % for dizygotic twins; the concordance rate of UC is about 6-16 % and 3 % respectively. C. There are significant ethnic differences in disease frequency. For instance, the prevalence in Ashkenazi Jews is much higher than that in other races, even though they share similar living environment in the same community. D. All genome-scanning linkage analyses detected some linkage loci, certain of which were subsequently confirmed by replication studies only involving certain...
chromosomes: NOD2 was consistently identified as the susceptibility gene for CD in recent years. E. Simulation studies on animal models have showed that transgenic mice or gene-knockout mice are subject to colitis similar to human IBD, and that spontaneous colitis or hapten-induced colitis manifests fairly different in different strains of mice\textsuperscript{[10-12]}. 

**Evidence of environmental contribution**
A. The concordance rate of IBD for monozygotic twins is much less than 100 %. The identical genotype with different phenotypes means that environmental factors take part in the pathogenesis of the disease\textsuperscript{[40,47]}. B. Intestinal bacteria are suggested as the main environmental contributions demonstrated by many evidences: antibiotic therapy can usually induce temporary remission for most IBD cases\textsuperscript{[13]}, diversion of faeces stream can make distal improvement in patients with CD\textsuperscript{[14,15]}, some studies suggested that certain strains of intestinal bacteria were associated with IBD\textsuperscript{[15,16]}, colonization with normal enteric bacterial flora was required for the occurrence of disease in animals with CD irrespective of the underlying defect\textsuperscript{[10-12]}. C. Smoking is likely to be associated with the progress of disease of IBD\textsuperscript{[17,18]}. D. Migrant epidemiological studies demonstrated that population of identical ethnic background, when lived in different communities, showed discordance\textsuperscript{[26-63]}. 

**IBD is not a disorder of simple mendelian inheritance**\textsuperscript{[22-25]}
Genetic disease of classic Mendelian model, which consists of Mendelian dominant and recessive genetic disorders, is a phenotype of single-gene defect and called single-gene disorder. IBD has previously been interpreted as genetic disease of Mendelian recessive model. But segregation analyses offered counter-evidence that IBD followed the principle of Mendelian inheritance. Parents of most IBD probands were healthy, frequency of siblings or children of the patients was much less than 50 %, the decline in frequency of affected second-degree relatives compared with first-degree relatives was greater than that predicted by autosomal dominant inheritance, in which the frequency was expected to decrease by 1/2 with each step. Incidence of IBD in children of affected spouses was sharply less than 100 % and a similar proportion of affected siblings and children of affected probands was inconsistent with autosomal recessive inheritance. Linkage analysis has detected several linkage loci that are distributed on a number of chromosomes.

**LINKAGE ANALYSIS**
It is very difficult to find the biochemical substances, which express qualitative difference between patients and healthy population by means of classical functional cloning. So linkage analysis, as the first step of positional cloning, may serve as a unique and practicable substitution for the time being. Figuring out genetic distance between marked loci and susceptibility gene by means of pedigree investigation and genotyping, then defining the approximate position of susceptibility gene in genomic map are the essential courses of linkage analysis. The dramatic progress of human genome project, which has located nearly 10 000 marker loci in genomic map, has greatly boosted positional cloning for complex genetic diseases. Epidemiological studies have identified striking genetic contributions to the etiology of IBD, but so far, studies with traditional biochemical methods have not yet identified the products with quantitative defects. Many investigators have turned to linkage analysis and have achieved great success. The important data from 38 original researches, which covered almost all articles in relation to IBD and linkage analysis that have been published since 1996, are listed in Table 1\textsuperscript{[26-60]}, and some aspects were reviewed as follows.

The common course of linkage analysis for IBD is: collecting families with affected sibling pairs (ASP) or affected relative pairs (ARP) ≥2 by strict ascertainment, genotyping of genome-wide or certain chromosomes according to microsatellite polymorphisms, figuring out multi-point maximal non-parametric LOD score (MLOD) and two-point LOD score by means of statistical software, inferring genetic distances of susceptibility genes to marker loci and locations in physical genome map, offering candidate genes for association analysis. The majority of investigations found certain suggestive linkage loci with various LOD score, but when defined according to different LOD thresholds, the locations or number of linkage loci were variable. In view of the traits of statistical software and quantity of subjects in most studies, we only displayed the results with MLOD ≥2.0 or 3.0, represented by ± and +. The chromosomes, on which the linkage loci strongly supported (MLOD ≥3.0) by at least one of 8 linkage analyses of genome-wide scanning located, include chromosomes 1, 3, 5, 6, 7, 12, 14, 16, 18 and 19, as well as chromosomes 4, 10, 17 and x with suggestive evidence (2.0 ≤MLOD <3.0). Although there was striking discrepancy among the genome-wide scans in respect of linkage loci, almost all studies detected more than 3 linkage loci. This shows that the pathogenesis of IBD is involved in multiple genes and manifests obvious genetic heterogeneity. Several loci were supported by relative more studies, such as 16q, 12q, 6p, and 3p. Because Hugot \textit{et al.}\textsuperscript{[26]} and Satsangi \textit{et al.}\textsuperscript{[27]} detected strong linkage evidences for chromosomes 16, 12, 6, 3 and 7 in 1996, subsequent studies mainly focused on these chromosomes. It can be seen from Table 1 that more evidences were offered for these loci, with the exception of 16q, simply because these loci were investigated by more studies. Some loci supported by certain genome-wide scans, such as 14q, 5q, 19p, likely to harbor susceptibility genes, were less investigated. Stratification studies demonstrated significant variances as to the degree and loci of linkage between families with severe IBD and those with only slight IBD, male patients and female patients, Jewish people and non-Jewish people, as well as between UC and CD. Some investigators examined families with CD patients only; others examined families with UC patients only, but most studies detected both families and those with mixed patients and compared the differences of linkage loci between the two groups. As shown in Table 1, there were some differences between UC and CD. The most striking is 16q12 (IBD1), which linked only with CD not UC in the majority of studies. This shows that CD and UC have some common susceptibility genes, as well as certain individual susceptibility genes. Three studies\textsuperscript{[22-24,48]} found that certain loci linked only with the families with early onset of CD. All subjects examined by the studies listed in Table 1 included Caucasian or Jewish patients from Europe, Australia and northern America, but no Mongolian patients. Three studies\textsuperscript{[25,29,42]} demonstrated significant differences between Jewish patients and non-Jewish patients. In respect of nationality of patients, it seems there are remarkable differences among American, English, German, Australian, Canadian, Italian, Dutch and Belgian. But Pauwela \textit{et al.}\textsuperscript{[49]} examined chromosomes 1, 3, 7, 12, 14 and 16 in Finnish patients and did not find linkage loci. Fisher \textit{et al.}\textsuperscript{[13]} found that some loci on chromosomes 6p, 1, 14 and 18 linked only with IBD of male sufferer. These results confirm the extensive genetic heterogeneity of IBD.

Linkage analysis is intended as an essential tactic to offer candidate genes for association analysis. We should focus our attention on the linkage loci containing some candidate genes, products of which have been suggested as pathogenic factors by other methods, as well as confirmed by subsequent replication studies. The loci meeting these conditions were briefly reviewed here.
Table 1 Data of linkage analysis

| Ref   | Author       | Year | Subject                  | Scope                  | Linkage loci for IBD | Linkage loci for CD | Linkage loci for UC |
|-------|--------------|------|--------------------------|------------------------|----------------------|---------------------|---------------------|
| R26   | Hugot JP     | 1996 | Caucasian CD             | Autosomal              | 16q(IBD1)+           | —                   | —                   |
| R27   | Satsangi J   | 1996 | Northern European IBD    | Autosomal              | ±, 12±, 3±           | 7±                  | —                   |
| R28   | Cho JH       | 1998 | American IBD*            | Genome                 | (3q±, 7p±, 10q±)     | 16±                  | —                   |
| R29   | M a Y        | 1999 | American CD              | Genome                 | ±, 7q±, 5q±         | —                   | 14q±, 17q±, 5q±    |
| R30   | Hampe J      | 1999 | European IBD             | Genome                 | ±, 7q±, 12q±, 3q±   | ±, 12±, 16±         | 4±, 7±, 16q±       |
| R31   | Duerr RH     | 2000 | American CD              | Genome                 | ±, 14q±              | —                   | —                   |
| R32   | Rioux JD     | 1998 | American IBD             | Genome                 | ±, 14q±, 15q±       | —                   | —                   |
| R33   | Curran ME    | 1998 | European IBD             | Genome                 | ±, 12q±              | —                   | —                   |
| R34   | Annese V     | 1999 | Italian IBD              | Genome                 | ±, 16q±              | ±                   | 16q±, 16q±         |
| R35   | Reimeire S   | 2000 | Belgian CD               | Genome                 | ±, 3,7,12,16±       | —                   | —                   |
| R36   | Dechoille B  | 2001 | European IBD             | Genome                 | ±, 6q±, 6p±         | —                   | —                   |
| R37   | Gavagnau A   | 2001 | IBD*                     | Genome                 | ±, 12q±              | —                   | —                   |
| R38   | Ohmen JD     | 1996 | American IBD             | Genome                 | ±, 16q±              | —                   | —                   |
| R39   | Parkes M     | 1996 | English IBD              | Genome                 | ±, 16q±              | —                   | —                   |
| R40   | Cavanaugh A  | 1998 | Australian CD            | Genome                 | ±, 6q±, 16q±        | —                   | —                   |
| R41   | Rizanz M     | 1998 | Northern European UC     | Genome                 | ±, 16q±, 16q±       | —                   | —                   |
| R42   | Porobasco P  | 2000 | Italian IBD              | Genome                 | ±, 16q±              | ±                   | 16q±               |
| R43   | Brant SR     | 2000 | American CD              | Genome                 | ±, 14q±, 16q±       | ±                   | —                   |
| R44   | Hampe J      | 1999 | Northern European IBD    | Genome                 | ±, 16q±, 16q±       | ±                   | —                   |
| R45   | Van Heel DA  | 2002 | European IBD             | Genome                 | ±, 16q±              | ±                   | —                   |
| R46   | Rioux JD     | 1998 | Toronto IBD              | Genome                 | ±, 6q±, 16q±        | ±                   | —                   |
| R47   | Paavola P    | 2001 | Finnish IBD              | Genome                 | ±, 16q±              | ±                   | —                   |
| R48   | Satsangi J   | 1999 | Northern European CD     | Genome                 | ±, 16q±, 16q±       | ±                   | —                   |
| R49   | Hampe J      | 1999 | Northern European IBD    | Genome                 | ±, 16q±, 16q±       | ±                   | —                   |
| R50   | Rioux JD     | 2000 | American CD              | Genome                 | ±, 16q±              | ±                   | —                   |
| R51   | Rioux JD     | 1998 | Toronto IBD              | Genome                 | ±, 6q±, 16q±        | ±                   | —                   |
| R52   | Scott J      | 1999 | Canadian IBD             | Genome                 | ±, 16q±, 16q±       | ±                   | —                   |
| R53   | Silverth II  | 1999 | Canadian CD              | Genome                 | ±, 16q±, 16q±       | ±                   | —                   |
| R54   | Hampe J      | 1999 | Northern European IBD    | Genome                 | ±, 16q±, 16q±       | ±                   | —                   |
| R55   | Hampe J      | 1999 | Northern European IBD    | Genome                 | ±, 16q±, 16q±       | ±                   | —                   |
| R56   | Duerr RH     | 1998 | Northern American IBD    | Genome                 | ±, 12q±, 16q±       | ±                   | —                   |
| R57   | Van Heel DA  | 2002 | European IBD             | Genome                 | ±, 12q±              | ±                   | —                   |
| R58   | Rioux JD     | 2001 | American CD              | Genome                 | ±, 12q±              | ±                   | —                   |
| R59   | Parkes M     | 2000 | American IBD             | Genome                 | ±, 12q±              | ±                   | —                   |
| R60   | Duerr RH     | 2002 | American IBD             | Genome                 | ±, 12q±              | ±                   | —                   |
| R61   | Rioux JD     | 2001 | American CD              | Genome                 | ±, 12q±              | ±                   | —                   |
| R62   | Vermeire S   | 2001 | Belgian CD               | Genome                 | ±, 12q±              | ±                   | —                   |

Note: CD, CD-only family; UC, UC-only family; IBD=UC+CD+mixed family; +, convincing linkage (LOD $>3.0$); ±, suggestive linkage (3.0$>LOD$ $>2.0$); —, no suggestive linkage (LOD $<2.0$); * including Jewish; † family from English, German and Dutch; ‡ family from Northern American, European and Australian; § family from French and Belgian; ¶ linkage only for Jewish; ‖ linkage only for IBD with early onset.

Chromosome 16 As shown in Table 1, 14 out of 25 related studies found linkage loci for CD with MLOD more than 2.0 on the chromosome, additionally, some loci with suggestive score (MLOD between 1.0 and 2.0) were detected. Only 3 studies found linkage loci with UC, furthermore, 2 of them also detected linkage with CD, and the other one merely examined UC families. It can be inferred from these studies that chromosome 16 contains susceptibility gene for CD rather than UC. Chromosome 16 is comparatively short, with 98 Mb of physical length, 130.8 cm of genetic distance, and has been spaced by about 200 microsatellite markers. The linkage loci suggested by the studies in Table 1 were distributed in most part of chromosome 16 (for instance, D16S409-419, D16S748-764, D16S41, D16S3136), but only pericentromeric region on 16q was the most consistent linkage region. The important candidate genes in the region are NOD2, CD11 integrins, CD19, Sialophorin, IL-4 receptor gene etc. NOD2 gene has been established as susceptibility gene to CD. It remains unanswered if there are other susceptibility genes in the chromosome. Hampe et al examined additional regions with high-density experiment using 39 microsatellite markers and found three-peak logarithm of odds (LOD) scores of 2.7, 3.2, and 3.1 on proximal 16p, proximal 16q, and central 16q, respectively. Taking account of the differences of suggestive markers, it is probable that there are other susceptibility genes for CD in the chromosome.

Chromosome 12 Six out of 19 studies found suggestive linkage loci in the chromosome, 4 of them for CD, and one for UC. Though the studies with suggestive MLOD are rare, several studies found linkage loci with slightly suggestive significance (MLOD between 1.0 and 2.0) with IBD, especially with UC. This may result from the fact that the sample sizes in most studies were not large enough; furthermore, they were predominantly consisted of CD families. Parkes et al examined 581 affected relative pairs, of which 252 were from CD-only families, 138 from UC-only families, and 191 from mixed families (the sample size was much larger than that in most of other studies, especially for UC), and found that MLOD at certain marker on chromosome 12 was 5.26 for all IBD, 3.91 for UC and 1.66 for CD. In summary, it is probable that...
The fact that the sample sizes were much smaller after stratifications and could not reach the threshold of statistical significance. It may be partly due to that for CD or UC alone and no LOD score for CD or UC alone reached 2.0 in all related studies. It should be noticed that LOD scores in 4 studies for all IBD were always higher than that for CD or UC alone and no LOD score for CD or UC alone reached 2.0 in all related studies. It may be partly due to the fact that the sample sizes were much smaller after stratifications and could not reach the threshold of statistical significance. It is very likely that there are some common susceptibility genes for CD and UC, but they probably confer slight genetic contribution to IBD, since some studies found linkage loci in the chromosome only with weakly suggestive LOD score[30,39,60]. The considerable linkage region was proximal 3p. The principal candidate genes in the region include CCR2, CCR5, IL-4RA, IL-5RA, lactotransferrin, IFN-α A2, cathelicidin antimicrobial peptide genes etc.

**Chromosome 5q** It was confirmed by only 3 studies involved in the chromosome. The linkage loci were located in 5q32-35, which happened to be in the region of cytokine-rich cluster and was called IBD5 in some studies. The main candidate genes are IL-3, IL-4, IL-5, IL-13, CSF-2 genes etc. The cytokines have been accepted as playing important roles in initiating IBD. So further investigations are needed.

**14q11(IBD4) and 19p13** They were suggested as linkage loci in some studies. 14q11 contains the immunoregulation members TCR-α/δ gene and 19p13 contains ICAM1, C3, TBX2A2 and LTB4H genes.

## ASSOCIATION ANALYSIS

Association analysis, as an essential step for positional cloning, was usually carried out by genotyping candidate genes selected by means of linkage analysis or other methods, for figuring out the frequencies of alleles and comparing the frequencies

| Ref year author | Subject | Main conclusion |
|-----------------|---------|-----------------|
| R65, 2001       | Europe CD, UC | A. Find P241S, R432R, R675W, G881R, IVS8-133delA in SCT, 980fs et al. |
| Hugot, JP       | American CD | A. 3020insC with CD |
| Ogura Y         | CD-GRR 3.0 at SHEM, 38.0 at HOM, 44.0 at CHEM |
| R67, 2001       | German, English CD | A. 3020insC with CD, not with UC |
| Hampe J         | UC | B. CD-GRR 2.6 at SHEM, 42.1 at HOM |
| Lesage S        | Europe CD, UC | A. Find 67 sequence variants, 9 of which gene frequency >5% |
| R69, 2002       | Europe CD, UC | A. R702W, G908R, 3020insC with CD, not with UC |
| Cuthbert AP     | A. Support gene-dosage effect |
| R70, 2002       | Dutch CD | A. 3020insC with CD, G2722C not with CD |
| Murillo L       | B. No association with clinical phenotype |
| R71, 2002       | German, Norwegian CD | A. R675W, G881R, 980fs with CD |
| Hampe J         | B. Especially with ileum CD |
| R72, 2002       | Canadian CD | A. R702W, G908R, 1007fs with CD, especially ileum CD |
| Vermeire S      | A. No difference between familial CD and sporadic CD |
| R73, 2002       | German UC, CD | A. 3020insC with CD, not with UC |
| Radimayr M      | B. Association with fistula, fibrostenosis, ileocecum resection |
| R74, 2002       | Japanese CD, UC | A. R675W, G881R, 3020insC not with CD or UC |
| Inoue N         | A. Support gene-dosage effect |
| R75, 2002       | Europe CD | A. R675W, G881R, 3020insC with CD |
| Vermeire S      | B. Not with effect of Infliximab |
| R76, 2002       | American CD | A. R702W, G908R, 1007fs with CD |
| Abrue MT        | B. With fibrostenosis |
| R77, 2002       | English CD | A. R702W, G908R, 1007fs with CD, especially ileum CD |
| Ahmad T         | A. 3020insC with early onset of CD |
| R49, 2002       | Europe CD | A. R702W, 1007fs with CD, linkage disequilibrium with P628S |
| Van heel DA     | B. Support gene-dosage effect |

**Note**: CD=Crohn disease; UC=ulcerative colitis; CD-GRR=CD-genotype relative risk; SHEM=simple heterogeneous mutation; HOM=Homzygous mutation; CHEM=complex heterogeneous mutation; P value is uniformly set at <0.05; Nomenclatures were not uniform among the studies, R675W=R702W, G908R=G908R, 1007fs=980fs=3020insC.
between IBD group and healthy control group to identify the specific allele. It has established that IBD is implicated in immune disorders. So studies have been centered on genes of NOD2/CARD15, HLA-II, cytokine, cytokine receptor and adhesion molecule.

**NOD2/CARD15 mutations**

Identification of NOD2 as susceptibility gene for IBD is supported by linkage analysis, association analysis and immunological function analysis. In this review, crucial information from 14 original studies on the relationships between IBD and NOD2 mutations are listed in Table 2 and comprehensively analyzed as follows. NOD1 is an intracellular protein composed of a N-terminal caspase recruitment domain (CARD), a centrally located nucleotide binding domain (NBD), and a leucine rich repeat (LRR) domain at its C-terminus which could activate nuclear factor \( \alpha \)B (NF-\( \alpha \)B) and also promote apoptosis[13]. NOD2 was identified by searching the public database for genes encoding similar proteins to NOD1. The gene happens to be located on chromosome 16q12, a domain called IBD1 supported by linkage analysis, association analysis and functional studies.

| Ref Year | Author | Subject | Region | Association with CD | Association with UC |
|----------|--------|---------|--------|---------------------|---------------------|
| R83 1995 | Duier RH | American UC | HLA-DR2 | DRB1*0405,0410,DQA1*03,DQB1*0401,0402+, (DQA1*0102,DRB1*1501,1302,DQB1*0602) - | DRB1*1601- |
| R84 1995 | Nakajima A | Japanese CD | DR, DQ, DP | DRB1*07+, DRB1*03- | |
| R85 1996 | Reishagen M | German CD | DRB1, DQA1, DQB1 | DRB1*07+, DRB1*03- | |
| R86 1996 | Herschbach D | French CD | HLA-I, HLA-II, TAP | DRB1*1302+, DRB1*04+ | DRB1*103+, DRB1*12+ |
| R87 1996 | Satsangi J | Europe IBD | DRB, DQB | DRB1, DQ1 | |
| R88 1996 | Danze PM | French CD | DRB1, DQ1 | DQB1*0501, DRB1*07, 01+ | DRB1*03- |
| R89 1997 | Bouma G | Dutch CD | DRB1*0701, C2*0802+, (DRB1*03) | |
| R90 1998 | Fernandez AM | Spanish UC | DRB1 | |
| R91 1999 | Lith M | Estonians UC | DRB1 | |
| R92 1995 | Seki SS | Japanese UC | HLA-I, HLA-II | Haplotype DRB3*0301/DQB1*1302+ | |
| R93 1999 | Yoshitake S | Japanese IBD | DQ, DR, DP | DQB1*0402+, DRB1*1502- | |
| R94 1999 | Stokkers PC | Meta-analysis | DR, DQ, TNF-\( \alpha \), LT-\( \alpha \) | DRB1*07,03,04+, (DR2,DR3)- | |
| R95 1999 | Hirv K | Estonians UC | DR, DQ, TNF-\( \alpha \), LT-\( \alpha \) | DRB1*0701, C2*0802+ | |
| R96 2001 | Seki SS | Japanese UC | HLA-I, HLA-II | |
| R97 2002 | Lantermann A | IBD* | DQA1 | DQA1+0201(German)+ | |
| R98 2002 | Orchard TR | Europe IBD | HLA-B, DR, TNF-\( \alpha \) | Uvetis with \( \beta \)P77, \( \beta \)S8,DRB1*0103 | |
| R97 2002 | Ahmad T | CD* | HLA-I, HLA-II, TNF-\( \alpha \), LT-\( \alpha \), HSP70 | DRB1*0701, C2*0802+ | |

**Table 3 Data of association analysis for HLA**

Note: UC=Ulcerative colitis; CD=Crohn disease; IBD=UC+CD; \( \alpha \)B=German, South Africa and South Korea IBD; \( \beta \)=white people CD; \( \gamma \)DRB1*04 is only associated with CD with no effect to cortexin; \( \beta \) value is uniformly set at \( P \leq 0.05 \); +, Positive association; -, Negative association.
studies have provided evidences that NOD2 is an intracellular receptor for bacterial pathogenic agents, and expresses only in monocytes. LRR of NOD2, after being activated by lipopolysaccharide (LPS), can trigger NF-κB signal pathway, which promotes the expression of certain proinflammatory cytokines. LRR can also promote apoptosis. So it is the most likely mechanism that the mutations in NOD2 either raise sensitivity of monocyte to bacterial pathogenic agents, with the result of overexpression of certain proinflammatory cytokines, or cause deficiency of apoptosis, leading to monocyte accumulation in intestinal mucosa and chronicity of the course. There are some questions about the relation between mutations in LRR and activity of NF-κB. Though high activity of NF-κB is always found in monocytes in lamina propria of intestinal mucosa from CD patients, it descends in cells with frame-shift mutation in LRR in vitro when stimulated by LPS. Functional study by Hugot et al demonstrated that expression of a NOD2 mutant form lacking the entire LRR region results in enhanced NF-κB activity, whereas the frame-shift mutation causing a truncated protein missing the final 33 amino results in low NF-κB activity. The potential explanation may be that the truncated protein leads to elevated NF-κB when stimulated by an untested bacterial LPS and that the frame-shift mutation may have a differential effect on caspase 9 induced apoptosis. To understand the mechanism how mutations in NOD2 confer susceptibility to CD, more functional analyses should be ingeniously performed. Identification of NOD2 was a great achievement in the history of exploring genetic susceptible factor for IBD. Detecting of the mutations may well have some clinical benefits for the prediction of onset risk, classification of disease, individualization of therapy and future gene therapy, but it should not be used as a tool for diagnosis since there is about 6-9 % overall allele frequency of the three single nucleotide polymorphisms (SNPs) in general population.

**HLA gene polymorphisms**

HLA gene is composed of three regions: HLA-I, HLA-II and HLA-III. HLA-I mainly includes HLA-A, HLA-B and HLA-C, and HLA-II mainly contains HLA-DR, HLA-DQ, HLA-DP. The primary immunity relative genes in HLA-III region are TNF-α, TNF-β, (LT-α), complement 2 (C2), complement 4(C4). Genes in the regions are highly polymorphic. HLA-II is expressed primarily in macrophages, dendritic cells and thymic epithelial cells, playing important parts in presenting exogenous antigen. Immunologic studies have shown strong evidences that IBD is closely associated with disturbance of Th lymphocyte subclass, and that the major environmental factor inducing immune disorder is intestinal bacterial flora. T lymphocytes accept enteric antigens presented by macrophages, so it is naturally viewed that sequence variants of HLA-II are likely to cause disorder of antigen presenting and result in imbalance of Th lymphocyte subclass. HLA genes located in IBD3 (6p13) were identified by linkage analysis, it is sensible to select HLA gene as candidate gene for association analysis. This review collected 18 studies published since 1995 and listed the main results in Table 3 [52, 77, 87-98]. Most studies published before 1995 usually typed HLA by examining serum HLA antigen through immunologic assay. The studies shown in Table 3 tried to identify HLA alleles by using reliable and precise molecular biological techniques, such as specific sequence primer polymerase chain reaction (PCR-SSP), specific sequence oligonucleotide probe assay (PCR-SSO) and gene sequencing.

It could be summarized from Table 3 that polymorphisms positively or negatively associated with UC or CD are mainly located in DRB1 or DQB1, which are the key regions to determine the polymorphism of peptide-binding cleft. The sequence variants in the regions could change the affinity for distinct antigen peptides. According to serum typing, results from more than two studies suggest that UC is positively associated with DR2, DR9 and negatively associated with DR4, and that CD is positively associated with DR7, DQ4, negatively with DR2, DR3. Further genotypings by molecular biological technique found that only DRB1*0103, DRB1*1501 and DRB1*1502 were associated with UC in more than two studies. Other variants in the regions have not been confirmed to be positively or negatively associated with UC or CD. Many investigators studied the association between polymorphism and clinical phenotype (p-ANCA, sex, extent, age of onset, site of disease, effect of certain medicine, complication, certain extraintestinal symptoms), but their conclusions were not consistent. The heterogeneity was obvious between UC and CD in respect of association with polymorphisms of HLA-II genes. The alleles associated with UC or CD were not associated with the others. On the contrary, DR2, positively associated with UC in some reports, was negatively associated with CD in other reports.

Stokkers et al [94] carried out a meta-analysis involving 29 pieces of related reports published from 1980 to 1999 (there were some overlaps between those and reports listed in Table 2). Taking a wider view of all these studies, we discovered a remarkable characteristic: the vast majority of studies discovered certain positively or negatively associated alleles, but all the suggested alleles showed significant discrepancy (there were always both evidences and counterevidences of the association as to any of the alleles among the studies). The contradiction cannot be explained by race heterogeneity because there are discrepancies both in white people and yellow people. It cannot be convinced that sequence variants in HLA-II gene are not associated with IBD and positive results are due to confusions either from linkage disequilibrium or from coincidences resulted from high polymorphisms. In view of the functional property of HLA-II and the widespread contradictions in association analysis, we consider that polymorphism in the regions, to some extent, plays a role in initiating IBD, but the involved alleles differ between different communities (in view of geographic situation, climatic condition and dietary culture) and even between individuals in the same community. The constituent characteristics (sort, ratio, total amount and time order of all antigens) of antigen compound (all kinds of antigens) derived from intestine in one community are distinct from that in another community. The antigen presenting cells (APC) containing certain HLA-II allele, only when disposing and presenting the antigen compound with matching constituent characteristic, can cause pathologic response. There are different predominant antigen compound in different communities, so the predominantly matching alleles implicated in IBD might be different among communities, and therefore no particular or unchanged antigen and HLA-II allele can take part in pathologic lesions in all IBD. Nevertheless, some of them may play a more dominant role in one community than in another community.

**Cytokine, cytokine receptor and adhesion molecule polymorphisms**

IL-1β, excreted mainly by microphages, up-regulates the expression of HLA-II, adhesion molecule and IFN-γ in an autocrine manner. It can also, through paracrine, promote activity of Th lymphocyte and play an important part in triggering immune response. IBD is regarded as the result of imbalance of Th lymphocyte subclass, thereby, IL-1β, IL-1β receptor (IL-1R), IL-1 receptor antagonist (IL-1RA), balance of IL-1β/IL-1RA may be associated with IBD. Up to date, many investigators have tried to explore association between IBD and gene polymorphism, but studies in recent years did not
find any positive results\[99-101\]. Nemetz et al found that IL-1β-511*2 allele was associated with the overexpression of IL-1β and descent of bone mineral density\[102\], and that IL-1β (+3953, 511) allele is associated with pathological course and patient’s condition\[103\]. Mwambe et al\[104\] examined the polymorphism of IL-1β, IL-1R and IL-1RA genes by TaqI, Pst I and VNTR and discovered IL-1R (TaqI-) allele frequency was significantly higher in white patients than that in white healthy controls and Negro patients, whereas IL-1R (Pst I-) allele frequency was higher in Negro patients than those in white patients. IL-1β and IL-1RA genes were not located in the region which was strongly supported by linkage analysis.

TNF-α is suggested as a pathogenic factor for IBD because its concentration is usually increased in intestinal mucosa of IBD patients, and therapy of anti-TNF-α antibody Infliximab has shown satisfactory effects on refractory IBD. The gene is located in IBD3 (6p13) which is supported by linkage analysis. Most studies in recent years discovered certain alleles were associated with IBD. Van Heel et al\[105\] found (-857C) TNF-α allele was associated with IBD. Sashio et al\[106\] found TNF-α (-308G/A, -238 G/A) allele was associated with UC. Mitchell et al\[107\] found that TNF-α (-308G/A) was associated with sclerosis cholangitis. Koss et al\[108\] found that different haplotypes of TNF-α gene were associated with the expression of TNF-α. Louis et al\[109\] discovered (-308) TNF-α was associated with certain clinical phenotypes of CD. Negoro et al\[110\] discovered (-1031C, -803A, -857T) TNF-α haplotypes of TNF-α gene were associated with the expression of TNF-α. These results show that sequence variants of TNF-α gene, especially (-308G/A) may take part in the pathogenesis of IBD by enhancing the expression of TNF-α and promoting activity and proliferation of Th lymphocytes.

IL-4, expressed mainly in Th2 lymphocytes, plays an important role in regulating the balance of Th lymphocyte subclasses and induces differentiation and proliferation of B lymphocytes or microphages, thereby it is regarded as a principal factor in initiating UC. IL-4 and IL-4 receptor (IL-4R) gene are separately located in 5q31-33 (IBD5) and 16q12 (IBD1) which are supported by linkage analyses. Klein et al\[111\] and Aithal et al\[112\] found that certain alleles of the genes were associated with CD. Peng et al examined IL-4 polymorphisms in Chinese people and found IL-4-RP2 allele frequency was obviously higher in UC patients than in healthy control, whereas RP1 allele frequency was higher in healthy control than in UC patients\[99\].

IL-10, mainly excreted by Th3 or Th2 lymphocytes, can suppress the expression of IL-12 and TNF-α in natural killer cells or microphages, restrain activity or proliferation of Th1 lymphocytes. The functional deficiency of IL-10 may be an important maintenance factor for chronicity of IBD. The fact that IL-10 gene knockout mice are subject to colitis similar to human IBD is a persuasive evidence. But studies in recent years did not discover any allele associated with IBD (allele IL-10 (-627, -711, -992, -891, -893, -894, -895) allele was associated with the down-regulation of IL-10 expression).

ICAM-1 plays an important role in regulating the homing of lymphocytes. Overexpression of ICAM-1 and significant lymphocyte infiltration have been found in intestinal mucosa of IBD patients. ICAM-1 gene is located in 19p13, which is supported by some linkage analysis. Yang et al\[115,116\] found that R241 allele was associated only with ANCA-positive UC. Contrarily, Braun et al\[117\] reported that ICAM-1 R241 allele and R/G241 heterogeneous mutation were much more frequent in UC patients than in healthy control irrespective of ANCA-positive or ANCA-negative.

Other investigators examined E-selection, L-selection, CCR2, CCR5, IL-6, NRAMP1 and IFN-γ genes and found they had no association with IBD\[95,118-121\].

**PROBLEMS AND PERSPECTIVES**

Taking a wide view of these studies, we could find extensive discrepancies, which are usually interpreted by the view that IBD is a genetic disease with widespread heterogeneity\[122\]. It means that the complicated clinical phenotypes of IBD are determined by interaction of multiple genes with environmental factors. Single gene contributes little to IBD, and only polygenic defects with corresponding conformation underlie the complicated phenotypes of IBD. One phenotype may be determined by more than one conformation models of polygenic defects. Nevertheless, we should take into account of other aspects to resolve the discordant results in linkage analyses. A. Entrance criteria and clinical classifications of subjects must be controlled more strictly and uniformly, since a minor mistake may influence the outcomes\[123,124\]. B. The microsatellite markers used by different investigators were not uniform, some investigators selected high-density markers, and others used somehow lower density markers. This could result in discrepancy conclusion. C. The sizes of sample were different among the studies, ranging from about 100 patients to more than 600 patients. Stratification studies performed using small sample sizes may cause false-negative error. Cavanaugh et al\[125\] carried out an international multicenter study, which involved 613 families from 12 study centers. By pooling of data sets, which were acquired from 12 independent centers using the same statistical method, despite the lack of convincing evidence for linkage based on data from individual center, they discovered unequivocal linkage for IBD on chromosome 16 (MLOD 5.79). D. The principle of linkage analysis is based on the view that crossing over in meiosis I is random and physical distance on chromosomes is necessarily in accord with genetic distance. The findings that significant association was found, but no linkage was suggested for the same subject group and the same loci demonstrated somehow theoretical disability of linkage analysis\[126\]. E. Other molecular biological mechanisms such as epigenetics may also play a role in initiating IBD\[127\]. In addition, if IBD is thought as a genetic disorder like familial adenomatous polyposis (certain mutations were inherited from parents, then somatic mutations were accumulated in certain cells such as macrophages or epithelial cells in intestinal mucosa or lymphoid tissue), all phenomena observed so far would not produce counterevidence. Today, linkage analysis has shed lights on genetic diseases such as diabetes mellitus, hypertension, asthma, Alzheimer’s disease and arteriosclerosis, as well as single-gene disorders. As for IBD, we have identified several linkage loci, which harbor a number of important candidate genes pending further confirmation by association analysis and functional analysis.

At present, about 30 candidate genes have been investigated by means of association analysis, and the majority of them either have no association with IBD or have not been confirmed by replication studies. With respect to HLA gene, though the majority of investigations discovered certain positively or negatively associated alleles, all the suggested alleles showed significant discrepancy. The three mutations in NOD2 gene have been consistently confirmed to be the independent susceptibility factors for CD in all-14 original studies except for one from a Japanese group, but how the variants can cause CD remains to be answered. Taking a wide view of all reports, which reached statistic significance, we discovered that frequencies of any alleles ever suggested by association analysis did not manifest a great absolute difference between patients and healthy controls. For instance, only about 20 % CD patients carried at least one of the three alleles of NOD2, whereas about 4-7 % healthy population also carried one of them. This shows that no allele ever studied demonstrates high specificity and sensitivity of the association with IBD and other
alleles need to be explored. It should be noted that the results of most studies, irrespective of positive or negative, did not absolutely ascertain the involvement of genes as susceptibility genes to IBD, since these could be confounded by a number of factors such as type I error or type II error caused by linkage disequilibrium or coincidence. In addition, there are many sequence variants in most genes, some of them are rare mutations and can only be properly analyzed in study of very large samples. Most investigators only detected variants in certain regions of the genes, instead of sequencing of whole gene of these alleles, therefore these results cannot represent the whole genes. The associations of some alleles with clinical phenotype of IBD have been detected by stratification study in many studies, but studies on the associations with the expression of cytokines involving the regulation of Th lymphocytes, were far less and more studies need to be carried out. The biochemical substances, which were ever suggested to be pathogenic factors for IBD, are of great variety, so it’s important to select proper candidate genes for association analysis. Since 1996, nearly 40 linkage analyses have identified several linkage loci in different chromosomes, such as IBD1, IBD2, IBD3, IBD4, and IBD5. Therefore, the genes located in such loci, and their products widely established as pathogenic factors for IBD, should be preferentially selected as candidate genes. Association analysis is an important method for unraveling the pathogenesis of IBD at gene level and will contribute tremendously to the understanding of IBD in the near future.

REFERENCES

1 Probert CS, Jayanthi V, Rampton DS, Mayberry JF. Epidemiology of inflammatory bowel disease in different ethnic and religious groups: limitations and aetiological clues. Int J Colorectal Dis 1996; 11: 25-28
2 Zheng JJ. Incidence of inflammatory bowel disease in Zheng JJ, Quo X, eds. Inflammatory bowel disease: basis and clinic. Beijing: Science Press 2001: 36-36
3 Binder V. Genetic epidemiology in inflammatory bowel disease. Dig Dis 1998; 16: 351-355
4 Hershbac D, Guwani-Akolkar B, Lesser M, Akolkar PN, Lin XY, Hershbad-Le Berne N, Breetagne JF, Katz S, Silver J. Anticipation in Crohn’s disease may be influenced by gender and ethnicity of the transmitting parent. Am J Gastroenterol 1996; 91: 2368-2372
5 Satsangi J, Grootochelten C, Holt H, Jewell DP. Clinical patterns of familial inflammatory bowel disease. Gut 1996; 38: 738-741
6 Tysk C, Lindberg E, Jarengot G, Fiedlerus-Myherd B. Ulcerative colitis and Crohn’s disease in an unsellected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. Gut 1988; 29: 990-996
7 Thompson NP, Driscoll R, Pounder RE, Wakefield AJ. Genetics versus environment in inflammatory bowel disease: result of a British twin study. BMJ 1996; 312: 95-96
8 Roth MP, Petersen GM, McElree C, Vadheim CM, Panish JF, Rotter JI. Familial empiric risk estimates of inflammatory bowel disease in Ashkenazi Jews. Gastroenterology 1989; 106: 1016-1020
9 Jayanthi V, Probert CS, Pinder D, Wicks AC, Maybery JF. Epidemiology of Crohn’s disease in Indian migrants and the indigenous population in Leicestershire O J Med 1992; 82: 125-138
10 Blumberg RS, Sauter C, Stover W. Animal models of mucosal inflammation. Their role to human inflammatory bowel disease. Curr Opin Immunol 1999; 11: 648-656
11 Kosiewicz MM, Nas CC, Krishnan A, Rivera-Nieves J, Masukal CM, Matsumoto S, Kazawa K, Cominelli F. Th1-type responses mediate spontaneous ileitis in a novel murine model of Crohn’s disease. J Clin Invest 2001; 107: 695-702
12 Wirtz S, Narabath MF. Animal models of intestinal inflammation: new insights into the molecular pathogenesis and immunotherapy of inflammatory bowel disease. Int J Colorectal Dis 2000; 15: 144-160
13 Mckay DM. Intestinal inflammation and the gut microflora. Can J Gastroenterol 1999; 13: 509-516
14 Shanahan F. Probiotics and inflammatory bowel disease: is there a scientific rationale? Inflamm Bowel Dis 2000; 6: 107-115
15 Masseret E, Boudreau J, Colombel JF, Neut C, Desruexsaux P, Joly B, Cortot A, Darjeufflle-Michaud A. Genetically related Escherichia coli strains associated with Crohn’s disease. Gut 2001; 48: 320-325
16 Sutton CL, Kim, JY, Yamanee A, Dalwadi H, Wei B, Landlers C, Targan SR, Braun J. Identification of a novel bacterial sequence associated with Crohn’s disease. Gastroenterology 2000; 119: 23-31
17 Cosnes J, Beaujolier L, Carbonnel F, Gendre JP. Smoking cessation and the course of Crohn’s disease: an intervention study. Gastroenterology 2001; 120: 1093-1099
18 Tysk C, Jarnert G. Has smoking changed the epidemiology of ulcerative colitis? Scand J Gastroenterol 1992; 27: 508-512
19 Salley J, Burda RL, Maybery JF, Probert CS, Roshan M, Samanta AK, Woods KL. Disease variations in Asians in Leicester. Q J Med 1993; 86: 263-269
20 Jayanthi V, Probert CS, Pollock DJ, Baithein SI, Rampton DS, Maybery JF. Low incidence of ulcerative colitis and proctitis in Bangladeshi migrants in Britain. Digestion 1992; 52: 34-42
21 Lander ES, Schork NJ. Genetic dissection of complex traits. Science 1994; 265: 2037-2040
22 Karban A, Eliakim R, Kantor SR. Genetics of inflammatory bowel disease. Inr Med Asoc J 2002; 4: 798-802
23 Laharie D, Debeugny S, Peeters M, Van Gossuin A, Gower-Rousseau C, Bclalche J, Fiasse R, Dupas JL, Lerebours E, Pottte S, Cortot A, Vermeire S, Grandbastien B, Colombel JF. Inflammatory bowel disease in spouses and their offspring. Gastroenterol 2001; 120: 816-819
24 Orthol M, Iselius L, Sorenson TN, Munkholm P, Langholz E, Binder V. Investigation of inheritance of chronic inflammatory bowel disease by complex segregation analysis. BMJ 1993; 306: 20-24
25 Kuster w, Paccol L, Purrmann J, Funk S, Majewski F, Paccol L, Purrmann J. The genetics of Crohn disease: complex segregation analysis of a family study with 265 patients with Crohn disease and 5,387 relatives. Am J Med Genet 1989; 32: 105-108
26 Hugot JP, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JJC, Beaugerie L, Naom I, Dupas JL, Van Gossuin A, Orholm M, Bonati-Pellie C, Weissbach J, Mathew CG, Lennard-Jones JE, Cortot A, Colombel JF, Thomas G. Mapping of a susceptibility locus for Crohn’s disease on chromosome 16. Nature 1996; 379: 825-833
27 Satsang J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell J, Jewell DP. Two-stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. N at Genet 1996; 19: 199-202
28 Cho JH, Nicolaie DL, Gold LH, Fields CT, Labuda MC, Rohal PM, Pickles MR, Qin L, Fu Y, Mann J, Kirschen BS, Jabs EW, Weber J, Hauauer SB, Bayles R, Brant SR, Identification of novel susceptibility lid for inflammatory bowel disease on chromosomes 1p, 3q, and 4p; evidence for epistasis between 1p and inflammatory bowel disease. Proc Natl Acad Sci U S A 1998; 95: 7502-7507
29 Ma Y, Ohmen JD, Li Z, Bentley LG, McElnree C, Pressman S, Targan SR, Fischel-Ghodsian N, Rotter JI, Yang H. A genome-wide search identifies potential new susceptibility loci for Crohn’s disease. Inflamm Bowel Dis 1999; 5: 271-278
30 Hampe J, Schreiber S, Shaw SH, Lau KF, Bridger S, Macpherson AJ, Cardon LR, Sakul H, Harris TJ, Buckler A, Hall J, Skokkes P, van Derventer SJ, Nurnberg P, Mirza MM, Lee JC, Lennard-Jones JE, Mathew CG, Curran ME. A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease on chromosomes 1p, 3q, and 4p; evidence for epistasis between 1p and inflammatory bowel disease. Proc Natl Acad Sci U S A 1998; 95: 7502-7507
31 Duer RR, Barmada MM, Zhang L, Pfutzer R, Weeks DE. High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. Am J Hum Genet 2000; 66: 808-816
32 Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Mcleod RS, Griffiths AM, Green T, Brattin TS, Stone V, Bulli SB, Bitton A, Williams CN, Greenberg CR, Cohen Z, Landers ES, Hudson TJ, Sominovitch KA. A genomewide search in Canadian families with inflammatory bowel disease reveals two new susceptibility loci. Am J Hum Genet 2000; 66: 1863-1870
culation of an inflammatory bowel disease genome search shows Male-specific linkage to the HLA region of chromosome 6. Eur J Hum Genet 2002; 10: 259-265.

Brant SR, Fu Y, Fields CT, Baltazar R, Ravenhill G, Pickles MR, Rohl PM, Mann J, Kirschner BS, Jabs EW, Bayless TM, Hanauer SB, Cho JH. American families with Crohn’s disease have strong evidence for linkage to chromosome 16 but not chromosome 12. Gastroenterology 1998; 115: 1056-1061.

Rioux JD, Daly MJ, Green T, Stone V, Lander ES, Hudson TJ, Steinhardt AH, Bull S, Cohen Z, Greenberg G, Griffiths A, McLeod R, Silverberg M, Williams CN, Simonovitch KA. Absence of linkage between inflammatory bowel disease and selected loci on chromosomes 3, 7, 12, and 16. Gastroenterology 1998; 115: 1062-1065.

Cavanaugh J. International collaboration provides convincing linkage replication for the IBD1 locus in inflammatory bowel disease. Am J Hum Genet 2001; 69: 926-934.

Kontula K. Genetic analysis in Finnish families with inflammatory bowel disease supports linkage to the IBD1 locus-a GISC Dis cover stud. Eur J Hum Genet 1999; 7: 567-573.

Karvonen AL, Julkunen R, Niemela S, Nurmi H, Farkkila M, Satsangi J, Lathrop GM, Bell JI, Jewell DP. Combined segregation and linkage analysis of inflammatory bowel disease in 16q34 candidate region for inflammatory bowel disease. Gastroenterology 2001; 121: 731-742.

Dannenberg AJ, Callen DF, Wilson SR, Stanford PM, Sraml ME, Barmada MM, Zhang L, Davis S, Fields CT, Baltazar R, Ravenhill G, Pickles AM, McLeod R, Silverberg M, Williams CN, Simonovitch KA. Evidence for linkage between Crohn disease and chromosome 16. Am J Hum Genet 2001; 69: 321-326.

Dennin LM, Fields CT, D'Adamo P, Scott R, Davies PG, Sarnik A, Smith LG, Davis S, Fields CT, Baltazar R, Ravenhill G, Pickles AM, McLeod R, Silverberg M, Williams CN, Simonovitch KA. Evidence for linkage to the IBD1 locus in inflammatory bowel disease. Gastroenterology 2001; 121: 731-742.

Silverberg MS. Evidence for linkage between Crohn disease and a locus near the major histocompatibility complex on chromosome 6 in a Canadian inflammatory bowel disease population. Gastroenterology 1999; 116: A20.

Hampe J, Shaw SH, Saiz R, Lysens N, Lantermann A, Mascheretti S, Lynch NJ, MacPherson AJ, Bridger S, van Deventer SJ, Stokkers P, Fischel-Ghodsian N, Silverberg M, Williams CN, Simonovitch KA. Evidence for linkage to the IBD1 locus in inflammatory bowel disease. Gastroenterology 2001; 121: 731-742.

Dennin LM, Fields CT, D'Adamo P, Scott R, Davies PG, Sarnik A, Smith LG, Davis S, Fields CT, Baltazar R, Ravenhill G, Pickles AM, McLeod R, Silverberg M, Williams CN, Simonovitch KA. Evidence for linkage to the IBD1 locus in inflammatory bowel disease. Gastroenterology 2001; 121: 731-742.

Andriulli A, Fortina P, Devoto M, Morton NE. Combined segregation and linkage analysis of inflammatory bowel disease in the IBD1 region using severity to characterise Crohn’s disease and ulcerative colitis. Eur J Hum Genet 1998; 6: 291-298.

Parkes M, Satsangi J, Lathrop GM, Bell J, Jewell DP. Susceptibility loci in inflammatory bowel disease. Lancet 1999; 354: 1588.

Cavanagh AJ, Callen DF, Wilson SR, Stanford PM, Sraml ME, Gorska M, Crawford J, Whitmore SA, Shlegel C, Fischel-Ghodsian N, Cottrill K. Genetic analysis in Finnish families with inflammatory bowel disease supports linkage to chromosome 3p21. Eur J Hum Genet 1999; 7: 328-334.

Cavanagh AJ. The IBD1 International Genetics Consortium. International collaboration provides convincing linkage replication in complex disease through analysis of a large pooled data set. Inflamm Bowel Dis 2000; 6: 165-170.

Ohmen JD, Yang HY, Yamamoto KK, Zhao HY, Ma Y, Bentley LG, Huang Z, Gerwesr S, Pressman S, McElreic C, Targan SR, Rotter J. Fischel-Ghodsian N. Susceptibility locus for inflammatory bowel disease on chromosome 16 has a role in Crohn’s disease, but not in ulcerative colitis. Hum Mol Genet 1999; 8: 1679-1683.

Parkes M, Satsangi J, Lathrop GM, Bell J, Jewell DP. Susceptibility loci in inflammatory bowel disease. Lancet 1999; 354: 1588.

Cavanagh AJ, Callen DF, Wilson SR, Stanford PM, Sraml ME, Gorska M, Crawford J, Whitmore SA, Shlegel C, Fischel-Ghodsian N, Cottrill K. Genetic analysis in Finnish families with inflammatory bowel disease supports linkage to chromosome 3p21. Eur J Hum Genet 1999; 7: 328-334.

Cavanagh AJ. The IBD1 International Genetics Consortium. International collaboration provides convincing linkage replication in complex disease through analysis of a large pooled data set. Inflamm Bowel Dis 2000; 6: 165-170.

Parkes M, Satsangi J, Lathrop GM, Bell J, Jewell DP. Susceptibility loci in inflammatory bowel disease. Lancet 1999; 354: 1588.

Cavanagh AJ, Callen DF, Wilson SR, Stanford PM, Sraml ME, Gorska M, Crawford J, Whitmore SA, Shlegel C, Fischel-Ghodsian N, Cottrill K. Genetic analysis in Finnish families with inflammatory bowel disease supports linkage to chromosome 3p21. Eur J Hum Genet 1999; 7: 328-334.

Cavanagh AJ. The IBD1 International Genetics Consortium. International collaboration provides convincing linkage replication in complex disease through analysis of a large pooled data set. Inflamm Bowel Dis 2000; 6: 165-170.

Parkes M, Satsangi J, Lathrop GM, Bell J, Jewell DP. Susceptibility loci in inflammatory bowel disease. Lancet 1999; 354: 1588.
Zheng CQ et al. IBD linkage and association analysis

63 Vermeire S, Satansgi J, Peeters M, Parkes M, Jewell DP, Vlaidinck R, Rutgeerts P. Evidence for inflammatory bowel disease of a susceptibility locus on the X chromosome. Gastroenterology 2001; 120: 834-840

64 Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gypsyak G, Morissette J, Weissenaer J. A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 1996; 380: 152-154

65 Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O’Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn’s disease. Nature 2001; 411: 599-603

66 Ogura Y, Bonen DK, Inohara N, Nicolea DL, Chen FF, Ramos R, Britton M, Moran T, Karilauski R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirscher BS, Hanauer SB, Nunez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn’s disease. Nature 2001; 411: 603-606

67 Hampe J, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeyer A, MacPherson AJ, Bridger S, van Deventer S, Forbes A, Nikolaus S, Lennard-Jones JE, Foelsch UR, Krawczak M, Lewis C, Schreiber S, Mathew CG. Association between insertion mutation in NOD2 gene and Crohn’s disease in German and British populations. Lancet 2001; 358: 1509-1512

68 Lesage S, Zouali H, Cezard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O’Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP. EPWG-IBD Group; EPIMAD Group; GETAID Group. CARD15 NOD2 mutational analysis and geno-type-phenotype correlation in 612 patients with inflammatory bowel disease. Am J Hum Genet 2002; 70: 845-857

69 Cuthbert AP, Fisher SA, Mirza MM, King K, Hampe J, Croucher PJ, Mascheretti S, Windsor J, Forbes A, Mansfield J, Schreiber S, Lewis CM, Mathew CG. The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. Gastroenterology 2002; 122: 867-874

70 Murillo L, Crusius JB, van Bodegraven AA, Alizadeh BZ, Pena AS. CARD15 gene and the classification of Crohn’s disease. Immunogenetics 2002; 54: 59-61

71 Hampe J, Grebe J, Nikolaus S, Solberg C, Croucher PJ, Mascheretti S, Jahnsten J, Mouts M, Klump B, Krawczak M, Mirza MM, Foelsch UR, Vatn M, Schreiber S. A modification of the association of NOD2 gene with clinical course of Crohn’s disease: a cohort study. Gut 2002; 50: 125-122

72 Reitsma PH, Tytgat GN, van Deventer SJ. HLA-DR and -DQ phenotypes in inflammatory bowel disease: a meta-analysis. J Autoimmun 1996; 9: 395-401

73 Hidiki K, Seyfarth M, Uibo R, Kull K, Salupere R, Latza U, Rink L, Bouma G, HLA-DR2 alleles in inflammatory bowel disease. Am J Hum Genet 2002; 71: 74-83

74 Minami Y, Tsuruno S, Kanai M, Seto H, Sato Y, Murakami S, Takayama Y, Nishida M. Association of MICA gene polymorphism with HLA-DR and -DQ alleles and subclinical markers. Clin Exp Immunol 1999; 116: 294-300

75 Yonekawa T, Takahashi S, Ogura Y, Inohara N, Nunez G, Cho JH. The contribution of NOD2 gene and Crohn’s disease. Am J Gastroenterol 2002; 97: 1462-1467

76 Kimura A, HLA-linked susceptibility and resistance genes in Crohn’s disease. Gastroenterology 1995; 109: 1462-1467

77 Danze PM, Colombel JF, Jacquot S, Leste MN, Heresbach D, Altepeko S, Khanassi S, Perichon B, Semana G, Charron D, Cezard JP. Association of HLA class II genes with susceptibility to Crohn’s disease. Gut 1996; 39: 69-72

78 Heresbach D, Alizadeh M, Reumaux D, Colombel JF, Delamare M, Danze PM, Gosselin M, Genetet B, Semana G. Are HLA-DR or TAP genes genetic markers of severity in ulcerative colitis? Autommum 1996; 9: 777-784

79 Bouma G, Oudkerk Pool M, Crusius JB, Schreuder GM, Hellemans HP, Meijer BU, Kostense PJ, Giphart MJ, Meuwissen SG, Pena AS. Are HLA-DR or TAP genes genetic markers of severity in ulcerative colitis? Am J Physiol 2002; 283: G1462-G1467

80 Hirt K, Seyfarth M, Uibo R, Kull K, Salupere R, Latza U, Rink L. Polymorphisms in tumor necrosis factor and adhesion molecule genes in patients with inflammatory bowel disease: associations with HLA-DR and -DQ alleles and subclinical markers. Scand J Gastroenterol 1999; 34: 1025-1032

81 Seki SS, Sugimura K, Ota M, Matsuzawa J, Katsuyama Y, Ishizuka K, Michuzuki T, Suzuki K, Yoneyama O, Mizuki N, Honma T, Inoko H, Asakura H. Stratification analysis of MICA polymorphism in inflammatory bowel disease. Am J Gastroenterol 2002; 97: 175-179

82 Vermeire S, Satansgi J, Peeters M, Parkes M, Jewell DP, Vlaidinck R, Rutgeerts P. Evidence for inflammatory bowel disease of a susceptibility locus on the X chromosome. Gastroenterology 2001; 120: 834-840

83 Inohara N, Koseki T, del Peso L, Hu Y, Yee C, Chen S, Carrio R, Merino J, Liu D, NJ, Nunez G. Nod1, an Apaf-1-like activator of caspase-9 and nuclear factor-kappaB. J Biol Chem 1999; 274: 14560-14567

84 Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G, Nod2, a Nod1 Apaf-1 family member that is restricted to monocytes and activates NF-KB. J Biochem 2001; 127: 4812-4818

85 Inohara N, Ogura Y, Chen FF, Muto A, Nunez G. Human Nod1 confers responsiveness to bacterial lipopolysaccharides. J Biochem 2001; 127: 2551-2554

86 Schreiber S, Nikolaus S, Hame J. Activation of nuclear factor kappa B in inflammatory bowel disease. Gut 1998; 42: 477-484

87 Zaree M, Singh PK, Irvine EJ, Sherman PM, McKay DM, Perdue MH. Monocyte/macrophage activation by normal bacteria and bacterial products: implications for altered epithelial function in Crohn’s disease. Am J Pathol 2001; 158: 1101-1109

88 Duer R, Neigt D. Molecularly defined HLA-DR2 alleles in ulcerative colitis and an antineutrophil cytoplasmic antibody-positive subgroup. Gastroenterology 1995; 108: 423-427
triplet repeat polymorphisms and HLA antigens associated with ulcerative colitis in Japanese. Tissue Antigens 2001; 58: 71-76

97 Lantermann A, Hanpej, Kim WH, Winter TA, Kidd M, Nagy M, Folsch UR, Schreiber S. Investigation of HLA-DPA1 genotypes as predictors of inflammatory bowel disease in the German, South African, and South Korean populations. Int J Colorectal Dis 2002; 17: 238-244

98 Orchard TR, Chuza CN, Ahmad T, Cheng H, Welsh KJ, Jewell DP. Uveitis and erythema nodosum in inflammatory bowel disease: clinical features and the role of HLA genes. Gastroenterology 2002; 123: 714-718

99 Peng Z, Hu P, Cui Y, Li C. Interleukin (IL)-1beta, IL-1 receptor antagonist and IL-4 gene polymorphisms in ulcerative colitis in the Chinese. Zhonghua Nei Ke Za Zhi 2002; 41: 248-251

100 Craggs A, West S, Curtis A, Welfare M, Hudson M, Donaldson P, Mansfield J. Absence of a genetic association between IL-1RN and HLA antigens associated with primary sclerosing cholangitis: no associations with interleukin 1beta gene polymorphisms in ulcerative colitis and Crohn disease in multiple populations from northeast England. Scand J Gastroenterol 2001; 36: 1173-1178

101 Donaldson PT, Norris S, Constantini PK, Bernal W, Harrison P, Williams R. The interleukin-1 and interleukin-10 gene polymorphisms in primary sclerosing cholangitis: no associations with disease susceptibility/ resistance. J Hepatol 2000; 32: 882-886

102 Nemetz A, Toth M, Garcia-Gonzalez MA, Zagoni T, Feher J, Pena AS, Tulassay Z. Allelic variation at the interleukin 1beta gene is associated with decreased bone mass in patients with inflammatory bowel diseases. Gut 2001; 49: 644-649

103 Nemetz A, Nosti-Escanilla MP, Molnar T, Kope A, Kovacs A, Feher J, Tulassay Z, Nagy F, Garcia-Gonzalez MA, Pena AS. IL1B gene polymorphisms influence the course and severity of inflammatory bowel disease. Immunogenetics 1999; 49: 527-531

104 Wantsemba O, Gaillard MC, Barkhuizen M, Pillay V, Berry SD, Dewar JB, Song E. Ethnic differences in allelic associations of the interleukin-1 gene duster in South African patients with inflammatory bowel disease (IBD) and in control individuals. Immunogenetics 2001; 52: 249-254

105 van Heel DA, Udalova IA, De Silva AP, McGovern DP, Kinouchi Y, Hull J, Lench NJ, Cardon LR, Carey AH, Jewell DP, Kwiatkowski D. Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NF-kappa B transcription factors. Hum Mol Genet 2002; 11: 1281-1289

106 Sashio H, Tamura K, Ito R, Yamamoto Y, Bamba H, Kosaka T, Fukui S, Sawada K, Fukuda Y, Tamura K, Satomi M, Shimoyama T, Furuyama J. Polymorphisms of the TNF gene and the TNF receptor superfamily member 1B gene are associated with susceptibility to ulcerative colitis and Crohn’s disease, respectively. Immunogenetics 2002; 53: 1020-1027

107 Mitchell SA, Grove J, Spurkland A, Boberg KM, Fleming KA, Day CP, Schrumpl F, Chapman RW. European Study Group of Primary Sclerosing Cholangitis. Association of the tumor necrosis factor alpha -308 but not the interleukin 10 -627 promoter polymorphism with genetic susceptibility to primary sclerosing cholangitis. Gut 2001; 49: 288-294

108 Koss K, Satsangi J, Welsh KI, Jewell DP. Cytokine (TNF alpha, LT alpha and IL-10) polymorphisms in inflammatory bowel disease and normal controls: differential effects on production and allelic frequencies. Genes Immun 2000; 1: 185-190

109 Louis E, Peeters M, Franchimont D, Seidel L, Fontaine F, Demolin G, Croes F, Dupont P, Davlin L, Omri S, Rutgeerts P, Belaieje J. Tumour necrosis factor (TNF) gene polymorphism in Crohn’s disease (CD): Influence on disease behaviour? Clin Exp Immunol 2000; 119: 64-68

110 Negoro K, Kinouchi Y, Hiwatashi N, Takahashi S, Takagi S, Satoh J, Shimosegawa T, Toyota T. Crohn’ disease is associated with novel polymorphisms in the 5'-flanking region of the tumor necrosis factor gene. Gastroenterology 1999; 117: 1062-1068

111 Klein W, Tromm A, Griga T, Frick H, Folwaczny C, Hocke M, Eitner K, Marx M, Duerig N, Epplen JT. Interleukin-4 and -4 receptor gene polymorphisms in inflammatory bowel diseases. Genes Immun 2001; 2: 287-289

112 Aithal GP, Day CP, Leathart J, Daly AK, Hudson M. A association of single nucleotide polymorphisms in the interleukin-4 and -4 receptor gene with Crohn’s disease in a British population. Genes Immun 2003; 2: 44-47

113 Aithal GP, Craggs A, Day CP, Welfare M, Daly AK, Mansfield JC, Hudson M. Role of polymorphisms in the interleukin-10 gene in determining disease susceptibility and phenotype in inflammatory bowel disease. Dig Dis Sci 2001; 46: 1520-1525

114 Klein W, Tromm A, Griga T, Frick H, Folwaczny C, Hocke M, Eitner K, Marx M, Runte M, Epplen JT. The IL-10 gene is not involved in the predisposition to inflammatory bowel disease. Electrophoresis 2000; 21: 3578-3582

115 Yang H. Analysis of ICAM-1 gene polymorphism in immuno logic subsets of inflammatory bowel disease. Exp Clin Immunogenet 1997; 14: 214-225

116 Yang H, Vora DK, Targan SR, Toyoda H, Beaudet AL, Rotter JI. Intercellular adhesion molecule 1 gene associations with immunologic subsets of inflammatory bowel disease. Gastroenterology 1995; 109: 440-448

117 Braun C, Zahn R, Martin K, Albert E, Folwaczny C. Polymorphisms of the ICAM-1 gene are associated with inflammatory bowel disease, regardless of the p-ANCA status. Clin Immunol 2001; 101: 257-360

118 Klein W, Tromm A, Griga T, Frick H, Folwaczny C, Hocke M, Eitner K, Marx M, Epplen JT. The polymorphism at position -174 of the IL-6 gene is not associated with inflammatory bowel disease. Eur J Gastroenterol Hepatol 2001; 13: 45-47

119 Koss K, Satsangi J, Welsh KJ, Jewell DP. Is interleukin-6 important in inflammatory bowel disease? Genes Immun 2000; 1: 207-212

120 Stokkers PC, de Heer K, Leegwater AC, Reitsma PH, Tytgat GN, van Deventer SJ. Inflammatory bowel disease and the genes for the natural resistance-associated macrophage protein-1 and the interferon-gamma receptor 1. Int J Colorectal Dis 1999; 14: 13-17

121 Hampe J, Herrmann B, Bridge S, MacPherson AJ, Mathew CG, Schreiber S. The interferon-gamma gene as a positional and functional candidate gene for inflammatory bowel disease. Int J Colorectal Dis 1998; 13: 260-263

122 Xia B, Crusius JBA, Meuwissen SGM, Pea AS. Inflammatory bowel disease definition, epidemiology, etiologic aspects, and immunogenetic studies. World J Gastroenterol 1998; 4: 446-458

123 Silverberg MS, Daly MJ, Moskovitz DN, Rioux JD, McLeod RS, Cohen Z, Greenberg GR, Hudson TJ, Smirnovitch KA, Steinart AH. Diagnostic misclassification reduces the ability to detect linkage in inflammatory bowel disease genetic studies. Gut 2001; 49: 773-776

124 Ghosh S. Linking genotype with phenotype in inflammatory bowel disease-Will we ever have reagent standard patients? Dis Markers 2000; 16: 167-171

125 Hampe J, Wieraker T, Nurnberg P, Schreiber S. Mapping genes for polygenic disorders: consideration for study design in the complex trait of inflammatory bowel disease. Hum Hered 2000; 50: 91-101

126 Schreiber S. Genetics of inflammatory bowel disease: a puzzle with contradictions? Gut 2000; 47: 746-747

127 Petronis A, Petroni rene E. Epigenetics of inflammatory bowel disease. Gut 2000; 47: 302-306

Edited by Yuan HJ and Wang XL