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Genomewide Search in Canadian Families with Inflammatory Bowel Disease Reveals Two Novel Susceptibility Loci

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The chronic inflammatory bowel diseases (IBDs)—Crohn disease (CD) and ulcerative colitis (UC)—are idiopathic, inflammatory disorders of the gastrointestinal tract. These conditions have a peak incidence in early adulthood and a combined prevalence of ~100–200/100,000. Although the etiology of IBD is multifactorial, a significant genetic contribution to disease susceptibility is implied by epidemiological data revealing a sibling risk of ~35-fold for CD and ~15-fold for UC. To elucidate the genetic basis for these disorders, we undertook a genomewide scan in 158 Canadian sib-pair families and identified three regions of suggestive linkage (3p, 5q31-33, and 6p) and one region of significant linkage to 19p13 (LOD score 4.6). Higher-density mapping in the 5q31-q33 region revealed a locus of genomewide significance (LOD score 3.9) that contributes to CD susceptibility in families with early-onset disease. Both of these genomic regions contain numerous genes that are important to the immune and inflammatory systems and that provide good targets for future candidate-gene studies.

Introduction

Crohn disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases (IBDs) associated with distinct clinical and pathological profiles. Specifically, CD is characterized by discontinuous, transmural inflammation potentially involving any part of the gastrointestinal tract, whereas in UC the inflammatory process is continuous and restricted to the mucosa of the large intestine.

Although both CD and UC are associated with altered expression of both proinflammatory and immunoregulatory cytokines within the intestinal mucosa, the patterns of cytokine alteration differ between the two conditions. Despite these and other differences, cumulative data garnered from epidemiological studies of these IBDs have revealed that relatives of individuals with either CD or UC are at increased risk for developing either form of IBD. Moreover, a number of IBD-affected individuals appear to manifest an overlap syndrome, known as "indeterminate colitis" (IC), which is characterized by features of both CD and UC. These observations suggest that at least some susceptibility genes will be shared by UC and CD.

The search for IBD-susceptibility genes has resulted in the identification of several loci with potential relevance to IBD etiology. These include *IBD1* (MIM 266600), a putative CD-susceptibility locus that maps to the pericentromeric region of chromosome 16 (Hugot et al. 1996), and *IBD2* (MIM 602458), mapped to a 41-cM region, on chromosome 12, surrounding the D12S83 marker and implicated in both CD and UC (Satsangi et al. 1996). The sibling relative risks (λ_s) for *IBD1* and *IBD2* are relatively low, having been estimated at 1.3 and 2.0, respectively. These localizations have been replicated in some but not all follow-up studies (Ohmen et al. 1996; Brant et al. 1998; Cavanaugh et al. 1998; Cho et al. 1998; Duerr et al. 1998; Rioux et al. 1998*a;* Annese et al. 1999; Hampe et al. 1999*a*) and, on the basis of available data, together would account for only a fraction of the heritability of IBD. Subsequent genomewide searches for susceptibility loci have also provided some suggestive evidence for linkage to other regions of the genome (Cho et al. 1998; Hampe et al. 1999*a;* Ma et al. 1999). The differences observed

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between the results of the various genomewide screens and follow-up linkage studies may be related to the difficulties in the detection of genes of modest effects in complex traits (Risch and Merikangas 1996) and to genetic heterogeneity. Specifically, this could be explained by a small number of alleles conferring modest susceptibility to IBD that are segregating at different frequencies in the given populations.

Considering (*a*) that not all IBD-susceptibility loci have been identified to date and (*b*) the possibility that they might be more easily identified in a population different from those previously studied, we decided to undertake a genomewide search for susceptibility loci in a Canadian population with IBD. This search began with identification of families in the Toronto region that had multiple siblings affected by IBD. Subsequently we examined the segregation of 312 microsatellite markers in 183 affected sibling pairs (ASPs) and in all available parents, using multipoint sib-pair analysis. This approach identified two novel loci of genomewide significance, in chromosomal regions 19p13 and 5q31-q33, and provided evidence that these loci confer susceptibility to IBD and CD, respectively, in this Canadian population. Both loci appear to have higher λ_s values in this population, compared with that in the previously reported loci *IBD1* and *IBD2*. Furthermore, both of these genomic regions contain many genes that are important to immune function and regulation and, given the inflammatory nature of these diseases, that represent interesting candidate genes.

Families and Methods

Families

Multicase families with two or more siblings affected by IBD were identified by review of clinical charts of all patients registered in the Mount Sinai Hospital Inflammatory Bowel Disease Centre database (McLeod et al. 1997) and The Hospital for Sick Children IBD database. Patients also were referred by physicians in the greater Toronto area. To confirm and update information obtained from these records, all patients were sent a questionnaire inquiring about the presence of a family history of IBD. Individuals identified as having one or more IBD-affected first-degree relatives were invited to participate and were asked for permission to contact other affected and unaffected family members. Endoscopic, histological, and radiological, reports, as well as clinical data, were obtained on all affected individuals, and these reports were reviewed by at least one of the authors of this study, for diagnosis verification based on standard criteria. Venous blood sampling was performed on affected individuals and their parents, and DNA was extracted by a salting-out procedure. Inclusion of families within the Jewish-ethnicity subgroup required a minimum of three grandparents of Jewish origin. Ethics approval for this study was given by the University of Toronto Ethics Committee. Written informed consent was obtained from all participants.

Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes from probands and family members of 158 White pedigrees. All parental DNA samples were genotyped when available: 140 families had both parents available, 17 families had one parent available, and 1 family had neither parent available. The genomewide scan, with an average intermarker spacing of 12 cM, was performed by use of a modified version of the Cooperative Human Linkage Centre Screening Set, version 6.0, that also included Généthon markers. These 312 loci were amplified with fluorescently labeled primers (Research Genetics), in separate PCRs, and the products were then multiplexed into panels by being pooled before electrophoresis on ABI 377 sequencers (PE Biosystems). Fluorescent genotyping gels were analyzed in an automated system developed at the Whitehead Institute/MIT Center for Genome Research (GENEHUNTER 2.0, Whitehead Institute for Biomedical Research/MIT Center for Genome Research). Further details of the genotyping system have been described elsewhere (Rioux et al. 1998*b*).

Both the region of suggestive linkage on chromosome 5 and the surrounding regions of poor information content were further investigated with additional microsatellite markers. Specifically, 34 markers were genotyped between markers D5S1470 and D5S1471, decreasing the average spacing between markers to ~3 cM in this 125-cM region. This higher-density mapping was performed on the original samples and on an additional 13 families (12 "CD only" families and 1 "mixed" family), for a total of 171 pedigrees analyzed. These additional families consisted of 15 ASPs where both members had CD and one mixed ASP where one sibling had CD and the other had UC.

Statistical Analysis

Multipoint sib-pair analysis of the data from the genomewide scan and from the higher-density mapping on chromosome 5 was performed by the MAPMAKER/SIBS functions implemented in GENEHUNTER 2.0 (Daly et al. 1998). All sib pairs from sibships with more than two affected individuals were counted but were conservatively down-weighted by a factor of $2/n$, where n is the number of affected individuals in the family (Suarez and Hodge 1979). Linkage analysis of different phenotypic subgroups was performed on the two chromosomes with the greatest evidence for linkage (i.e., chromosomes 5 and 19). Specifically multipoint sib-pair analysis was performed by use of four different phe-

Table 1

Summary of Families and ASPs Included in the Genomewide Screen

		No. of ASPs				
Pedigree Type	No. of Families	Both Sibs with CD	Both Sibs with UC	One Sib with CD and One Sib with UC	One Sib with IC and One Sib with Either UC or CD	Total
CD only	104	116	...	...	...	116
UC only	20	...	20	...	...	20
Mixed	34	8	...	26	13	47
Total	158	124	20	26	13	183

notypic categories: IBD, UC, CD, and CD16. In the first, all individuals with CD, UC, or IC were designated as affected; in the second, only individuals with UC were designated as affected; in the third, only individuals with CD were designated as affected; and, in the fourth, only individuals with CD were designated as affected and only families with at least one affected sibling diagnosed at age ⩽16 years were included. Exclusion mapping was also performed with the GENEHUNTER 2.0 package; exclusion was declared at a LOD score of ⩽−2.

To establish appropriate thresholds for suggestive and significant genomewide linkage for these particular data sets, simulations were performed by generation of artificial genotype data with identical family structures (GENSIM computer program designed by Mark J. Daly [unpublished data]). These simulations matched our data sets with respect to marker density, marker informativeness, individuals genotyped, affected status, and fraction of missing data. Specifically, 500 whole-genome screens were generated at the genome-scan marker density (1 marker every 10 cM, throughout). In this fashion, the genomewide thresholds for suggestive and significant linkage were determined to be LOD scores of 1.9 and 3.3, respectively. Since we tested only one phenotype—that is, the all-inclusive "IBD"—no correction for multiple testing was necessary. Subsequent to the higher-density mapping of the 5q31-q33 region, we simulated 1,000 whole-genome screens at the higher marker density (1 marker every 2 cM, throughout), to assess the significance of the linkage results. Only 8 simulated genome scans of 1,000 ($P < .01$) had values that exceeded our observed value of 3.94 on chromosome 5. Correction for multiple testing was necessary, since we examined the suggestive regions for different hypotheses: we examined five different subgroups based on phenotype, age at diagnosis, and ethnicity and performed limited correlation tests. Specifically, to search for interactions either between the two novel loci described herein or between these loci and *IBD1* and *IBD2,* we examined the correlation between family-based multipoint sib-pair linkage scores computed by GENEHUNTER 2.0, as described elsewhere (Cox et al. 1999). These analyses (subgroup analysis and correlation tests) should not be considered as whole-genome scan tests, because they were done only on regions of suggestive linkage. Even if all five tests were to be considered independent, our result is still likely to be seen in less than .05 genomewide scans—the accepted threshold for declaration that linkage is significant.

To examine the possibility of obvious segregation distortion affecting our linkage results on either chromosomes 5 or chromosome 19, we used the transmission/disequilibrium test (Spielman and Ewens 1996). Specifically, a single trio was selected at random from each family, and the alleles at each marker were examined for excess transmission. No single marker or pair of markers had any demonstrable transmission-ratio distortion.

The analysis of the subgroup of families with early-onset cases (i.e., families that would be expected to have greater disease severity and familial component) was done by selection of families in which the youngest age at diagnosis was ⩽16 years. This cutoff value was chosen after observation that the distribution of the youngest age at diagnosis in a family was much better fit by two Gaussian distributions, with means at 13.1 and 20.2, than by a single Gaussian distribution ($\chi^2 = 19.8$, 3 df; $P < .0002$). The Gaussian fitting was done by an implementation of the Levenberg-Marquardt method, as described elsewhere (Press et al. 1992).

Results

To further define the genetic basis of IBD, we performed a genomewide scan on 158 families comprising 183 ASPs with IBD (table 1). These families included 116 ASPs with CD, 20 ASPs with UC, and 47 mixed ASPs (i.e., CD, UC, and/or IC). In the majority of families, parental-DNA samples were available for genotyping: 88.6%, 10.8%, and 0.6% had DNA from both parents, one parent, or neither parent, respectively. All ASPs and available parents were genotyped with 312 microsatellite markers covering the genome at an average spacing of 12 cM.

Multipoint sib-pair analysis of the data was then performed; the results are shown in figure 1. On the basis of simulation experiments, the genomewide threshold for suggestive linkage (i.e., the score expected to occur

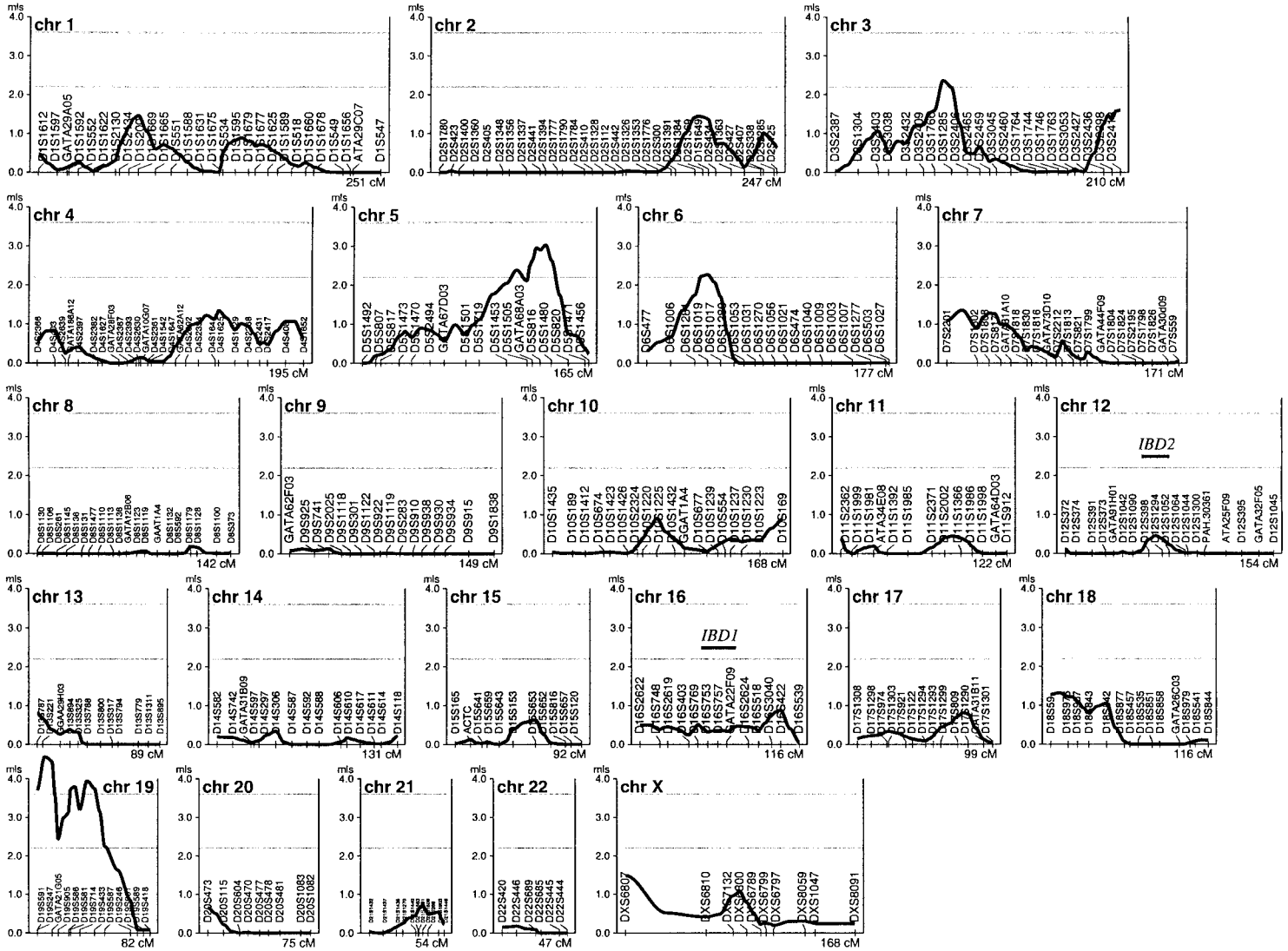

Figure 1 Multipoint sib-pair analysis results for the IBD genome scan. Multipoint LOD scores were calculated by use of the MAPMAKER/SIBS functions implemented in GENEHUNTER 2.0. The vertical axis indicates the LOD score along the length of each chromosome, and the tick marks indicate the positions of the microsatellite markers. The two horizontal lines indicate the published genomewide thresholds (Lander and Kruglyak 1995) for suggestive (LOD score 2.2) and significant linkage (LOD score 3.6). The horizontal bars on chromosomes 12 and 16 indicate the position of the previously reported IBD-susceptibility loci *IBD1* and *IBD2* (Hugot et al. 1996; Satsangi et al. 1996).

one time at random in a genomewide scan) was determined to be a LOD score of 1.9. Specifically, 500 whole-genome screens were generated to simulate the actual pedigrees and genotype information. This calculated threshold was quite similar to the recommended threshold, estimated in the limit of an infinitely dense genetic map, of a LOD score of 2.2 (Lander and Kruglyak 1995). When either of these values was used, four regions in our genomewide search surpassed the threshold for suggestive linkage to IBD. As shown in figure 1 and table 2, these loci localized to chromosomal regions 19p13, 5q31-33, 3p, and 6p, with peak LOD scores of 4.6, 3.0, 2.4, and 2.3, respectively. Moreover, the evidence for linkage at the chromosome 19 locus was greater than a LOD score of 3.6, the threshold for genomewide significance (Lander and Kruglyak 1995). In addition, significant marker-allele sharing between siblings was observed at this 19p13 locus (proportion of sibs sharing 0, 1, and 2 alleles: $Z_0 = .08$, $Z_1 = .50$, and $Z_2 = .42$, respectively). The region on chromosome 19p13 thus appears to represent a novel IBD-susceptibility locus, with an apparent λ_s of 2.2 (95% confidence interval 1.6–2.8).

Among the chromosomal loci showing suggestive linkage to IBD, the 5q31-q33 region was of particular interest, because the region spans the major immunoregulatory cytokine-gene cluster. Accordingly, we genotyped the families in the initial scan, together with 13 newly ascertained families, for 34 additional microsatellite markers in and around this region. Multipoint sib-pair analysis was then performed on the data, with the families being stratified by clinical phenotype (i.e., CD vs. UC). Although, with the additional markers and families (table 3), there was a decrease in the linkage score for the all-inclusive IBD phenotypic category, analysis of the UC and CD categories clearly demonstrated that linkage to this locus was almost exclusively found in the CD families.

Furthermore, previous studies of CD have revealed that early age at onset is associated with increased disease severity and increased heritability (Polito et al. 1996). Accordingly, the chromosome 5 linkage data were examined to specifically assess the subgroup of CD16 families. As shown in table 3, the 50 families in this subgroup had a substantially higher peak LOD score: 3.9. This peak, centered around marker D5S2497, showed 70% sharing between affected siblings ($Z_0 = .05$, $Z_1 = .50$, and $Z_2 = .45$), and an apparent λ_s of 2.8 (95% confidence interval 1.7–4.7). On the basis of simulation experiments at this higher marker density, the genomewide threshold for significant linkage (i.e., the score expected to occur .05 times in a genome scan) was determined to be a LOD score of 3.3. Our observed LOD score therefore exceeds this threshold, as well as the significance threshold based on an infinitely dense map (Lander and Kruglyak 1995), and thus reveals that the chromosomal region 5q31-33 contains a locus for susceptibility to early-onset CD. By contrast, the evidence for linkage on chromosome 19p13 was not enhanced by an identical age–based stratification of families (table 3).

Table 2

Summary of Genome-Scan Results

Chromosomal Region	Interval of Peak Linkage	Peak LOD Score
3p	D3S1766–D3S1285	2.4
5q31-33	D5S816–D5S1480	3.0
6p	D6S1281–D6S1019	2.3
19p13	D19S591/D5S247–GATA21G05	4.6

Table 3

Summary of Significant Linkage Results, according to Phenotypic Category

Chromosome and Phenotypic Category	No. of Pedigrees	Peak LOD Score
5:		
IBD	171	2.4
UC	24	0.1
CD	122	3.0
CD16	50	3.9
19:		
IBD	158	4.6
UC	24	2.9
CD	110	3.0
CD16	46	1.5

In view of both the increased frequency of IBD and increased familial risk for these diseases within the Jewish population (Yang et al. 1993), the linkage data were also analyzed by stratification of the families as Jewish or non-Jewish. As indicated in table 4, the results of this analysis revealed that the evidence for linkage in the chromosome 5q31-33 and 19p13 regions was common to both the Jewish and non-Jewish families. In addition, since previous reports have raised the possibility of epistatic interactions between IBD-susceptibility loci (Cho et al. 1998), we searched for evidence of interlocus interactions, by evaluating correlations between within-family linkage scores. No significant positive or negative correlations, either between the novel loci on chromosomes 5 and 19 or between either the loci reported herein and the *IBD1* and *IBD2* loci reported elsewhere (data not shown), were detected.

It should be noted that the other two suggestive loci, although showing weaker evidence of linkage to IBD, are located near previously reported loci of suggestive linkage. Specifically, the chromosome 3p linkage peak in the present study is located ~10 cM from a locus seen in a U.K. population (Satsangi et al. 1996), and

Table 4

Contribution of Ethnic Subgroups to Significant Linkage Results

Chromosome and Phenotypic Category	No. of Pedigrees	Peak LOD Score
5:		
CD16, Jewish	24	2.2
CD16, non-Jewish	26	2.1
19:		
IBD, Jewish	44	2.2
IBD, non-Jewish	114	2.7

the chromosome 6p linkage peak in the present study is located ~15 cM from a locus seen in a western-European population (Hampe et al. 1999*b*).

Finally, to assess whether the *IBD1* and *IBD2* loci are contributing to the IBD susceptibility in the population that we studied, we performed exclusion mapping. These analyses demonstrate that we can exclude almost all of chromosome 12, for loci of even modest effects (i.e., $\lambda_s > 2.0$), but that, because of excess sharing—that is, 55% ($Z_0 = .23$, $Z_1 = .45$, and $Z_2 = .32$)—in the *IBD1* region, we can exclude, on chromosome 16, only loci conferring $\lambda_s > 4$, suggesting that *IBD1* (reported $\lambda_s \sim 1.3$) could be playing a role in this Canadian population with IBD.

Discussion

Disease susceptibility in IBD is complex and most certainly involves the interaction between environmental factors and multiple genes. Although our knowledge of the former remains limited, a number of genomewide linkage studies have begun to increase our knowledge of the latter. Indeed, the first two completed studies revealed significant susceptibility loci, now known as "*IBD1*" and "*IBD2,*" on chromosomes 16 and 12, respectively (Hugot et al. 1996; Satsangi et al. 1996); these loci were reported to contain genes that contributed to the susceptibility to CD and IBD, respectively, albeit of modest effect ($\lambda_s \leq 2$). Not all subsequent studies were confirmatory, and some provided evidence for additional susceptibility loci (Hampe et al. 1999*a;* Ma et al. 1999; Cho et al. 1998; Rioux et al. 1998*a*). Considering that the overall λ_s values for CD and UC are relatively large (35 and 15, respectively) and that the reported loci could not explain the entire genetic susceptibility, we decided to examine a Canadian population with IBD.

The results of this genomewide search of 183 Canadian ASPs revealed the presence of a novel locus, on chromosomal region 19p13, that confers susceptibility to IBD. These results also provided evidence for three loci suggestive of linkage that are located on 5q31-q33, 3p, and 6p. The 5q31-q33 region was further studied, with additional genetic markers, and stratification of the data set according to clinical phenotype demonstrated that the linkage evidence was almost exclusively within the CD subset. In an attempt to further understand the contribution of this suggestive locus to CD susceptibility, we examined the 50 families with early-onset disease, since this has been suggested as an approach to definition of a subgroup of patients with increased relative risk (Lander and Schork 1994). This approach was used successfully in determination of the linkage to breast cancer (Hall et al. 1992) and is supported by previous studies of CD, which indicated that early onset of disease was associated with increased disease severity and increased heritability (Polito et al. 1996). Indeed, the present analysis revealed a 5q31-q33 locus, of genomewide significance, that contributes to CD susceptibility primarily in families with early-onset cases. It is noteworthy that, although none of the five previously reported genomewide studies have provided evidence for linkage to the 19p13 locus, the studies by Cho et al. (1998) and Ma et al. (1999) have shown some evidence for linkage to the 5q31-q33 region, with LOD scores of 1.2 and 2.2, respectively.

The identification of 5q31-33 and 19p13 as potential locations for IBD-susceptibility genes is of particular interest in view of the biology of the genes known to map within these regions. The chromosomal segment corresponding to 5q31-33 includes many cytokine genes, including those encoding interleukins 3–5 and 13, as well as colony-stimulating factor 2. Although the etiology of IBD remains unknown, a role for immunological dysregulation is widely supported by the results of numerous studies on the immune-cell populations and on cytokine expression patterns within IBD-affected intestinal tissue (Kmiec 1998). In fact, a clustering of loci on the syntenic region in mice, for numerous models of autoimmunity, has suggested that genes in this region are implicated in the Th1/Th2 balance (Vyse and Todd 1996); the perturbation of which may also be important in development of IBD. This is further supported by recent linkage data derived from a murine model of colitis, which has localized an IBD-susceptibility gene to this same syntenic region on mouse chromosome 11 (Mahler et al. 1999).

As does the 5q31-33 region, the chromosome 19p13 region also contains a number of genes representing strong candidates for IBD; these include the genes encoding intercellular adhesion molecule 1 (ICAM1), complement component 3 (C3), the thromboxane A2 receptor (TBXA2), leukotriene B4 hydroxylase (LTB4H), and the janus protein tyrosine kinases TYK2 and JAK3. The relevance of any of these genes to IBD etiology remains to be elucidated, but at least some of the genes already have been implicated in IBD pathogenesis. For example, modest associations have been reported between selected

ICAM1 and C3 polymorphisms and IBD (Elmgreen et al. 1984; Yang et al. 1995); and interference with the TBXA2-mediated (Takao et al. 1997) and LTB4H-mediated (Hawkey et al. 1992) inflammatory pathways has been shown to be of potential therapeutic value in experimental IBD models. The janus kinases also represent logical candidates for involvement in IBD, in view of their critical roles in the coupling of cytokine stimulation to cell activation (Onishi et al. 1998).

Higher-density mapping will be necessary to follow up the suggestive linkages to chromosomes 3 and 6 loci identified here, but it is interesting to note that the linkage peaks reported herein are located near previously reported suggestive loci. In particular, the linkage peak on chromosome 6 lies ~15 cM proximal to the major histocompatibility complex (MHC) class II region, a region widely recognized as playing an integral role in predisposition to immunological disease. Importantly, a recent study has described linkage to this chromosome 6 region (Hampe et al. 1999*b*), and a large metanalysis of the results derived from 29 different studies also has reported both CD and UC to be associated with specific class II alleles (Stokkers et al. 1999). It remains to be determined, however, whether the susceptibility is actually conferred by an MHC gene or by a gene in disequilibrium with this complex.

In conclusion, this study reports two novel susceptibility loci: a locus on chromosome 5q31-33, which appears to confer particular susceptibility to a subset of early-onset CD-affected individuals; and a locus on chromosome 19p13, which confers susceptibility to both CD and UC. Both loci appear to have higher λ_s values in this population, compared with the previously reported loci *IBD1* and *IBD2*. Further work will be necessary to elucidate the causative genes and the mechanisms by which they contribute to the genetic susceptibility to IBD.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Cooperative Human Linkage Center, http://lpg.nci.nih.gov/CHLC

Généthon, http://www.genethon.fr

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for *IBD1* [MIM 266600] and *IBD2* [MIM 602458])

Whitehead Institute for Biomedical Research/MIT Center for Genome Research, GENEHUNTER software distribution site, http://www.genome.wi.mit.edu/ftp/distribution/software/genehunter (for GENEHUNTER 2.0)

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