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An SNP linkage scan identifies significant Crohn's disease loci on chromosomes 13q13.3 and, in Jewish families, on 1p35.2 and 3q29

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Abstract

Inflammatory bowel disease (IBD) is a complex genetic disorder of two major phenotypes, Crohn's disease (CD) and ulcerative colitis (UC), with increased risk in Ashkenazi Jews. Twelve genome-wide linkage screens have identified multiple loci, but these screens have been of modest size and have used low-density microsatellite markers. We, therefore, performed a high-density single-nucleotide polymorphism (SNP) genome-wide linkage study of 993 IBD multiply affected pedigrees (25% Jewish ancestry) that contained 1709 IBD-affected relative pairs, including 919 CD–CD pairs and 312 UC–UC pairs. We identified a significant novel CD locus on chromosome 13p13.3 (peak logarithm of the odds (LOD) score = 3.98) in all pedigrees, significant linkage evidence on chromosomes 1p35.1 (peak LOD score = 3.5) and 3q29 (peak LOD score = 3.19) in Jewish CD pedigrees, and suggestive loci for Jewish IBD on chromosome 10q22 (peak LOD score = 2.57) and Jewish UC on chromosome 2q24 (peak LOD score = 2.69). Nominal or greater linkage evidence was present for most previously designated IBD loci (IBD1–9), notably, IBD1 for CD families at chromosome 16q12.1 (peak LOD score = 4.86) and IBD6 in non-Jewish UC families at chromosome 19p12 (peak LOD score = 2.67). This study demonstrates the ability of high information content adequately powered SNP genome-wide linkage studies to identify loci not observed in multiple microsatellite-based studies in smaller cohorts.

Keywords

Introduction

Inflammatory bowel disease (IBD) (MIM # 266600) is a disorder involving chronic intestinal inflammation and is composed of two major phenotypes, Crohn's disease (CD) and ulcerative colitis (UC). CD is characterized by discontinuous transmural inflammation involving any portion of the gastrointestinal tract, with the ileum and colon most commonly affected.[1] In UC, inflammation is limited to the mucosa, and always involves the rectum and, to a variable extent, the colon in a continuous manner.[1] In approximately 10% of individuals, confirmed IBD limited to the colon cannot be clearly classified as UC or CD and the IBD is labeled as 'indeterminate colitis.'[2] Phenotypic characteristics vary between both CD- and UC-affected individuals, particularly in the location and extent of involved gut, presence of extraintestinal manifestations (approximately 25% of persons with IBD have arthritis, uveitis, pyoderma gangrenosum, erythema nodosum or primary sclerosing cholangitis) and for CD, development of fistulizing or stricturing complications. Other immune-mediated disorders are more frequent among IBD patients, notably, ankylosing spondylitis, asthma, psoriasis and multiple sclerosis.[3] IBD may occur at any age, although the peak age at onset is the third decade of life. Both CD and UC have a prevalence of 100–200 per 100 000 in the United States.

Epidemiological studies have long suggested that IBD is a complex genetic disorder: 10–30% of patients have a family history of IBD, monozygotic (MZ) twin concordance is significantly greater than dizygotic (DZ) twin concordance (20–50% for CD and 6–16% for UC versus 0–4% for CD and 0–5% for UC, respectively), and IBD risk is two- to ninefold greater in persons of Ashkenazi Jewish ancestry.[4–7] λ_s, the sibling recurrence risk ratio, has

been estimated as 24.7 for IBD, 36.5 for CD and 16.6 for UC.[8] Both CD and UC appear genetically related as the cross-disease relative risk was observed to be 3.85 with a CD proband and 1.72 with a UC proband.[9]

To date, 12 published genome-wide screens and follow-up studies have identified loci with genome-wide linkage evidence and confirmation in single or combined studies (*IBD1–9*) on chromosomes 16q21 (CD), 12p13–q24, 6p21–23, 14q11–12 (CD), 5q31 (CD), 19p13–q13, 1p36, 16p12 and 3p26–14, respectively.[10–22] An additional six loci have been detected with suggestive evidence of linkage in one study and replication evidence in one or more additional studies on chromosomes 2q23–35 (UC), 3q25–28, 4q22–31, 7p13–q21, 11p15–12 and Xp22–21.[22] Notably, defined risk alleles/haplotypes have been identified for the *IBD1*(*NOD2*), *IBD3* (human leukocyte antigen) and IBD5 (5q cytokine cluster) loci.[23–26] Despite the tremendous successes of IBD linkage studies to date, several fundamental challenges remain for IBD genetics: (1) replication evidence for loci is highly inconsistent and candidate regions for the majority of loci without established risk haplotypes span large chromosomal regions; (2) individual genome-wide linkage screens have been relatively small (six with less than 100 pedigrees and only one with over 200 pedigrees) and none have had 80% power to identify loci with λ_s 1.5; (3) a screening density range of 9–14 cM, frequently using the same marker panels, has likely left important regions unexamined; (4) with no screen of more than 64 Jewish ancestry IBD pedigrees, no loci explain the disproportionate Ashkenazi Jewish risk and (5) no UC locus has been established, perhaps because even the largest IBD genome screen had less than 120 UC relative pairs.

The NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) IBD Genetics Consortium (NIDDK-IBDGC) was established in 2002 in large part to assemble the large number of IBD pedigrees required to identify overlooked genetic loci, identify loci to account for IBD phenotypes and population subgroups, confirm and narrow down candidate regions and make progress toward disease allele identification. Here, we report the results of a new genome-wide linkage scan, the first scan to use less than 1 cM density single-nucleotide polymorphism (SNP)-based markers, on nearly 1000 well-characterized IBD multiply affected families, one-quarter of them being of Jewish ancestry.

Results

The NIDDK IBDGC assembled DNA samples on 1016 independent multiplex IBD pedigrees, containing two or more IBD, CD or UC informative affected relative pairs with diagnoses confirmed, for a whole-genome SNP-based linkage study. The DNA samples were genotyped using a total of 6008 SNP markers, part of the Illumina Linkage IVb Marker Panel, at the SNP Center at the Center for Inherited Disease Research (Baltimore, MD, USA), with 5912 SNPs released by the SNP Center. We dropped an additional 42 SNPs based on mean allele frequency of less than 0.05 and/or Hardy–Weinberg equilibrium *P*<0.0001. We used the program Merlin to check for Mendelian inconsistencies and to identify genotypes associated with an excessive number of observed recombinations.[27] As a result, an additional 120 genotypes were removed from the analysis. Taken together, the Mendelian consistency rate for this project was 97.55%. Following data cleaning, linkage analysis was performed using the program Merlin on 5750 markers. Twenty-three pedigrees were dropped in the analyses by Merlin as the relative pairs were too distantly related (beyond first cousin pairs), leaving 993 IBD pedigrees analyzed containing 1709 total informative affected IBD relative pairs (Table 1), including CD–CD pairs (*n* = 919), UC– UC pairs (*n* = 312) and mixed pairs (*n* = 478; including CD–UC pairs and pairs with one or both affected relatives diagnosed with indeterminate colitis). Separate analyses, as detailed in Table 1, were performed stratified by Jewish ancestry and for CD–CD pairs ('CD pairs') and UC–UC pairs ('UC pairs').

Merlin can automatically define linkage disequilibrium (LD) clusters that take the LD structure into account (data not shown) and then conduct analysis after modeling marker–marker disequilibrium. When using a cutoff R^2 of 0.2, the analysis modeling LD clusters eliminated a few initially identified linkage peaks, including a very strong signal (non-parametric linkage (NPL) = 10.2) on chromosome 21. The results with three phenotypes, IBD, CD and UC, with the full cohort and with the stratified data set by Jewish origin are summarized in Figures 1a, b and c. The significant linkage findings in 'all ethnicities', Jewish and non-Jewish populations, have been summarized in Table 2.

Significant linkage findings were detected on chromosomes 16q12.1 (logarithm of the odds (LOD) score = 4.86) and 13q13.3 (LOD score = 3.98) for the CD phenotype for the entire cohort, and on chromosomes 1p35.2 (LOD score = 3.5) and 3q29 (LOD score = 3.19) for Jewish pedigrees. No non-Jewish-only significant linkage findings were detected in this study for the criteria of a genome-wide significance level of 0.05, as determined by permutation testing (Table 2).

The strongest linkage evidence was observed at the *IBD1* locus on chromosome 16. We observed a very sharp linkage peak at 49.23Mb (rs745230) on chromosome 16 for the CD (peak LOD score = 4.86) compared with the IBD (peak LOD score = 1.00) phenotype. The NOD2 gene maps adjacent to this peak (between 49.29 and 49.32 Mb). The second strongest signal was observed on chromosome 13q13.3 with the CD phenotype with a genome-wide significance level of 0.01 (Table 2). Because it is known that insufficient adjustment for LD correlation between linkage markers can potentially inflate the LOD score,[28] we used a more stringent R^2 cutoff of 0.1 to conduct the linkage analysis for the CD phenotype (including both Jewish and non-Jewish families) on chromosome 13; the results varied slightly (from 3.98 to 3.87). We compared the LD structure clusters among the Jewish and non-Jewish groups and the clusters appeared to be very similar, particularly in the region where we found the chromosome 13q13.3 peak. To date, this is the first report of an identification of this novel susceptibility locus on chromosome 13q.[22] There was no linkage evidence for the UC phenotype at this locus (Figure 1). In addition, signals on chromosomes 1p35.2 and 3q29 both met the criteria of genome-wide significance levels less than 0.05 in Jewish families but not in non-Jewish families (Table 2). Our screen identified two suggestive loci for Jewish families on chromosome 2q24 (maximum LOD score = 2.69 at marker rs1990760 for the UC phenotype) and chromosome 10q22 (maximum LOD score = 2.57 at marker rs585895 for the IBD phenotype). The only novel linkage signal observed in the non-Jewish families was, for the first time in UC families, to the IBD6 locus at chromosome 19p12 (LOD score = 2.67), although the genome-wide significance level for this signal was 0.194.

The peak LOD scores obtained by this linkage scan in previously identified linkage regions of *IBD1*–9 are shown in Table 3 for all families. Nominal evidence of linkage greater than LOD score of 1.5 was observed in most of these regions, with exceptions of IBD4, IBD5 and IBD9.

Discussion

In this study, we identified genomic regions demonstrating significant evidence for linkage to CD on chromosomes 16q12.1 and 13q13.3, and suggestive evidence for linkage to UC on chromosome 19p12. Furthermore, we found significant linkage between Jewish CD and loci on chromosomes 1p35.2 and 3q29. The reliability of this study is, in part, demonstrated by the strong confirmation of the *IBD1* locus with a peak LOD score of 4.86 in CD–CD pairs and no evidence in UC–UC pairs. This is consistent with *IBD1*, and its putative gene, NOD2, established as CD risk factors. The maximal LOD score at rs745230 is only 60.8kb

away from NOD2, highlighting the fine resolution achieved in this study. Interestingly, we continue to observe a 'shoulder' to the overall linkage peak on the p-arm of chromosome 16 (Figure 1a). This increased linkage score is observed in both Jewish and non-Jewish CD and is not due to decreased information content in the centromere. In support of a *NOD2*-independent CD signal in this region, Hampe et al. reported a haplotype-based association[29] for CD (*IBD8*), near D16S3068 with a peak significance level of 0.002, with modest enrichment stratified by *NOD2* genotype.[30] The present linkage findings are consistent with the presence of additional IBD susceptibility alleles residing on the p-arm of chromosome 16, a possibility that will be examined in further studies.

A major finding of the present study is the identification of a novel, significant CD linkage peak on chromosome 13p13.3. In our prior combined analysis of microsatellite-based screens, we observed only modest evidence for linkage (MLOD = 0.5) in this region.[31] An increased information content (0.89 in the present screen versus 0.76 in the prior study) for the 1 LOD confidence interval between 31.53 and 37.65Mb combined with a significantly larger number of pedigrees in the CD phenotype analysis (574 families) likely contributed to the novel linkage discovery. For the $R^2 > 0.2$ analysis, we included all genotyped markers, as none in this region demonstrated an $R^2 > 0.2$ with each other. Our linkage results were robust to the LD between genotyped markers in the region; limiting markers to R^2 0.1 had minimal effect on the linkage signal. Taken together, this would indicate that as yet unidentified CD-specific susceptibility alleles reside in this region. The most notable gene that maps within the 95% confidence interval is the RFXAP gene, which encodes the 36kb regulatory subunit of regulatory factor X, a nuclear protein complex that is a specific transactivating factor for major histocompatibility complex class II promoters. RFXAP gene mutations are one cause of major histocompatibility complex class II deficiency found in the immunodeficiency syndrome, bare lymphocyte syndrome type II.[32] Other genes, listed in the NCBI database, that map within the 95% confidence interval may be found in Supplementary Table 1.

The established IBD associations in *NOD2, IBD5, IL23R* and *ATG16L1* do not account for the several-fold increased IBD prevalence observed in Jewish populations.[33] One would anticipate that if directly causative variants are identified, their frequency might be significantly greater in the Jewish population. However, this has not been observed thus far for any of the established risk alleles. Furthermore, association evidence has not been observed for the *IBD5* risk haplotype in Jewish CD; however, it has not yet been established if the causal variants at *IBD5* have been identified.[34] Taken together, this would indicate that major susceptibility alleles of particular importance in the Jewish population have yet to be identified. Toward these ends, our findings of significant linkage of Jewish CD to chromosomes 1p35.2 and 3q29 are of particular importance. Given the allelic architecture of the IBD association within the *IL23R* gene on chromosome 1p31.3, involving an uncommon, strongly protective allele at Arg381Gln, the *IL23R* associations are unlikely to generate a significant linkage signal. Consistent with this, the *IL23R* gene is located outside the 1.5 LOD confidence interval for the observed linkage peak, and therefore the linkage signal at chromosome 1p35 most likely represents an IBD susceptibility allele independent of *IL23R*.

A comparison of the top linkage findings from the present study (see Table 2) with our recent ileal CD genome-wide association study[33] identified (in addition to NOD2-associated SNPs for the *IBD1* chromosome 16 peak) only one SNP with association evidence of potential interest (that is, $P < 0.0001$) within the chromosomes 1p35.2 linkage peak (rs10914850, $P = 7.5 \times 10^{-5}$) and one SNP within the 3q29 linkage peak (rs6787480, $P = 9 \times 10^{-5}$). Conversely, we did not observe any CD linkage peaks among chromosomal segments that correspond to any of the multiple, highly significant novel associations observed among recent CD genome-wide association studies with the exception of

NOD2.[33,35–38] Notably, for the three most well-replicated associations after NOD2—the IBD5 risk haplotype, IL23R and ATG16L1—the established risk haplotypes or missense variants associated are common in the population and thus the inheritance of risk alleles may frequently come from different founders within a given pedigree weakening potential linkage signals.[33,34]

The findings of the present study support the concept that genome-wide linkage and association studies represent complementary approaches to gene identification. SNP-based genome-wide linkage studies in large, well-powered cohorts, such as in the present study, provide improved information content and power to identify novel linkage regions, such as those identified presently at chromosome 13p13.3, prioritize the significance of linkage peaks and improve localization information in regions identified in prior studies. The present identification of genome-wide significant linkage signals in regions lacking established IBD associations highlights the complementary information provided by linkage and genome-wide association studies. Genotyping platforms used in genome-wide association studies focus on common SNPs with minor allele frequencies greater than 5%. Assaying of less common, untyped variations can be achieved by multimarker haplotype analyses, but are still dependent on the limitations of the HapMap data set. In contrast, linkage approaches are powered to identify uncommon alleles conferring high genotype relative risks. Association in linkage areas may also not be found should the risk within a gene region be secondary to multiple risk alleles that are not in LD with each other. The presence of significant linkage on chromosomes 1p and 3q in Jewish CD cohorts may reflect the contribution of uncommon variation relatively unique to the Ashkenazi Jewish population. Subsequent studies will involve a careful integration of all sources of information, together with resequencing to identify uncommon genetic variation contributing to IBD.

Patients and methods

Families with IBD and at least two affected individuals with confirmed IBD diagnoses (CD, UC or indeterminate colitis) were recruited through the IBD genetic research studies at the University of Chicago Genetics Research Center, the Meyerhoff Inflammatory Bowel Disease Genetics Research Center at the Johns Hopkins Hospital, the University of Pittsburgh Genetics Research Center, the University of Montreal Genetics Research Center, the University of Toronto Genetics Research Center and the Cedars-Sinai Genetics Research Center in Los Angeles. Informed consent for a genetic research study for IBD was obtained from all study subjects. All diagnoses of CD, UC or indeterminate colitis were confirmed by physician reviewers from each center from primary chart reviews of endoscopy, radiology and pathology data as meeting standard diagnostic criteria.[39–41] Forty-three percent of pedigrees included were previously studied in one of six microsatellite linkage reports from North America,[12,14–17,20] and results of a majority of these pedigrees were included in a single genome-wide microsatellite mega-analysis we described previously.[31] Study subjects were self-identified as white race. A pedigree was defined as Jewish if two or more affected relatives were of Jewish ancestry (two or more grand-parents Jewish).

Genotyping was performed at the SNP Center at the Center for Inherited Disease Research, Baltimore, MD, USA, using the Illumina Linkage IVb Marker Panel on an Illumina BeadLab system. We computed marker allele frequencies using data from one individual randomly chosen from each pedigree. The NPL method[42] implemented in MERLIN, version 0.10.2, was employed.[27]

Kong and Cox[42] propose testing the null hypothesis given $\delta = 0$, where δ is the extent of deviation from IBD sharing. The Kong and Cox LOD$_{all}$ statistics were computed since it is known that the score 'all' provides more information when relative pairs included in a data

set are not independent, and that 'all' outperforms 'pairs' when the level of heterogeneity underlying a complex trait is relatively high.[43] Initially, we computed multipoint NPL scores without taking LD structure into account and then repeated the analysis after modeling marker–marker disequilibrium using Merlin to eliminate the potential for false-positive results; Merlin can automatically define LD clusters using the user-provided marker map and genotype data. We used an R^2 cutoff of 0.2 to generate LD clusters for the Jewish and non-Jewish populations separately. The detailed algorithm that clusters tightly linked markers and uses haplotype frequencies to model marker–marker LD was described by Abecasis and Wigginton.[28]

For the most significant linkage signal detected by this scan (other than the chromosome 16 peak that accounts for the *NOD2* locus), we also used a more stringent R^2 of 0.1 to conduct the linkage analysis in CD families. All SNP allele frequencies were estimated separately for the Jewish and non-Jewish cohorts.

We estimated empirical *P*-values by using simulation under the null hypothesis, with 10,000 replicates with Merlin. For each replicate, the original phenotype was used and a new data set, with the same SNP allele frequencies, marker order, genetic distances between markers, as well as missing data pattern was generated. Therefore, the 'significant signals' obtained through simulation are chance findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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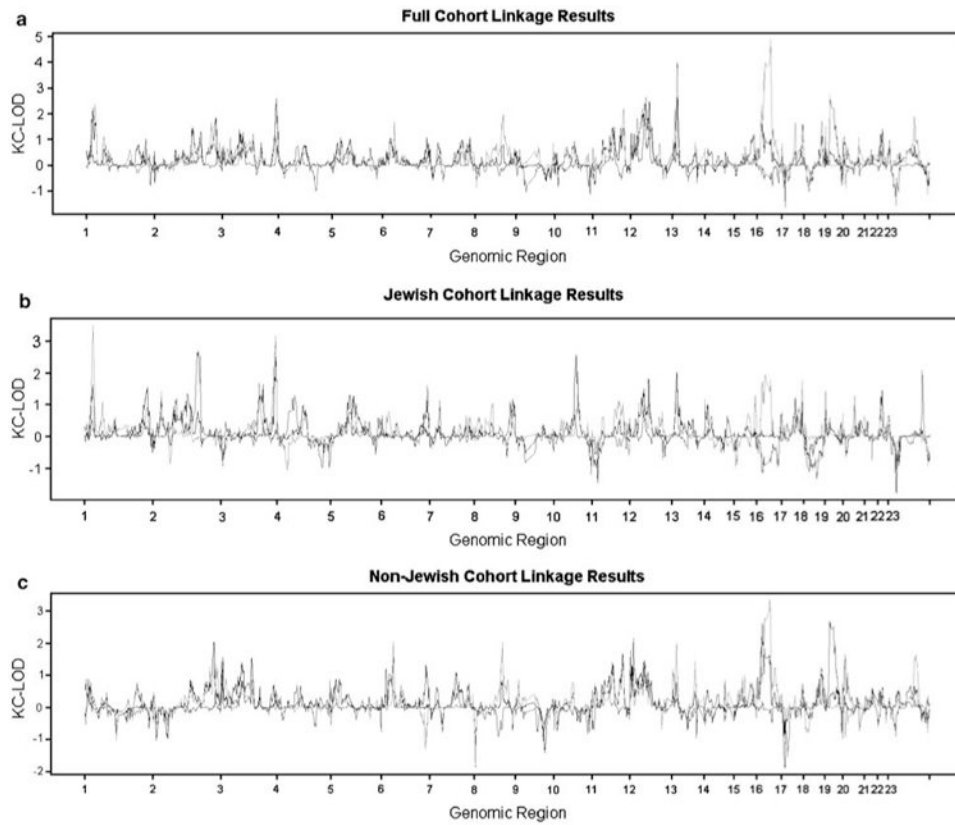
Figure 1.
Lod scores by chromosomal regions for the full cohort (**a**), Jewish pedigrees (**b**), and Non-Jewish pedigrees (**c**). KC-LOD, Kong and Cox LOD score; black solid line, all IBD pairs; red solid line, CD–CD pairs; blue solid line, UC–UC pairs. See online version for color Figure.

Table 1

Family characteristics used in this analysis

Analytic set	Informative families per Merlin	Affected relative pairs total (sibling, grandparent, avuncular, 1/2 sibling, cousin)	Average number of members with DNA submitted for genotyping (range)
All families			
All IBD pairs	993	1709 (1049, 77, 371, 5, 207)	4.33 (2–22)
CD pairs	574	919 (623, 21, 174, 3, 98)	4.40 (2–22)
UC pairs	215	312 (185, 16, 78, 0, 33)	4.74 (2–16)
Jewish families			
All IBD pairs	244	374 (228, 32, 68, 0, 46)	4.11 (2–13)
CD pairs	137	196 (140, 11, 26, 0, 19)	4.03 (2–8)
UC pairs	52	68 (29, 8, 16, 0, 15)	4.67 (2–13)
Non-Jewish families			
All IBD pairs	650	1160 (699, 36, 274, 4, 147)	4.45 (2–22)
CD pairs	386	638 (418, 7, 137, 3, 73)	4.56 (2–22)
UC pairs	140	210 (131, 7, 56, 0, 16)	4.83 (2–16)

Abbreviations: CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.

Table 2

Significant findings by phenotype and ethnicity

Band	Cohort	Phenotype	Marker	Position[a]	Observed LOD[b]	Sim-P[c]	Boundary of 95%	confidence interval
16q12.1	All	CD	rs745230	49227683	4.86	0.002	rs189378	rs1123428
13q13.3	All	CD	rs20411	35129488	3.98	0.010	rs1329344	rs668103
1p35.2	Jewish	CD	rs555920	30728957	3.5	0.024	rs204057	rs12408030
3q29	Jewish	CD	rs2986	194013904	3.19	0.050	rs2067078	rs903196

[a] Genomic location on chromosome (Build 35.1).

[b] Observed logarithm of the odds score.

[c] P-value determined by using simulation.

Table 3

LOD scores in the regions harboring previously identified IBD loci

IBD locus	Band	Phenotype	Peak LOD of this scan
IBD1	16p13.1–q12.2	CD	4.86
IBD2	12p12–q13	UC	1.86
IBD3	6p23–p21.1	IBD	1.62
IBD4	14q11–q12	IBD	0.21
IBD5	5q31–q35	UC	0.62
IBD6	19p13.2–q13.2	IBD	1.46 (CD)
			2.67 (UC)
IBD7	1p36.2–p32	IBD	2.17
IBD8	16p12	IBD	1.73
IBD9	3p26	IBD	0.58

Abbreviations: CD, Crohn's disease; IBD, inflammatory bowel disease; LOD, logarithm of the odds; UC, ulcerative colitis.