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Additional evidence of linkage between Crohn's disease and a putative locus on chromosome 12

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Purpose: The inflammatory bowel diseases (IBD), Crohn's disease (CD), and ulcerative colitis (UC) are chronic intestinal disorder of unknown etiology. Genetic factors play an important role in the pathogenesis of these diseases, but with a complex pattern of inheritance. A number of genome-wide scans have identified several putative susceptibility loci for both CD and UC, including a locus on chromosome 12 reported in a set of British families. We aim to evaluate the linkage between CD or UC and this chromosome 12 locus in an independent set of U.S. Caucasian families (36% being of Ashkenazi Jewish origin). **Methods:** Microsatellite markers along chromosome 12 spaced at approximately 10 cm intervals were used to test the putative loci in CD only families (65 sib pairs from 46 families). Regions with positive linkage for CD were then tested in a panel of UC and mixed families (44 sib pairs from 29 families). Two point linkage analysis was performed with SIBPAL. Multipoint linkage analysis was carried out with MAPMAKER/SIBS. **Results:** We observed evidence of linkage between the region on chromosome 12 and Crohn's disease, because there was a significant excess of allele sharing in CD sib pairs (π = 0.62, p = 0.0004 from two-point linkage; and logarithm of the odds score (LOD) = 2.0 from multipoint linkage). However, we did not observe the same linkage in UC and mixed families (p = 0.48; not significant [ns]). Conclusion: Our data provided further evidence that the region on chromosome 12 is likely to contain a gene predisposing to CD. ***Genetics in Medicine*, 1999:1(5):194–199.**

Key words: *Linkage, inflammatory bowel disease, chromosome 12, Crohn's disease, genetic study*

Chronic inflammatory bowel disease (IBD) presents as two major clinical forms, Crohn's disease (CD) and ulcerative colitis (UC). These are chronic debilitating disorders of the intestine, which occur mostly in young adults with an estimated prevalence of 200–300/100,000 in the United States (for reviews see Calkins and Mendeloff[1]). According to estimates of the Crohn's and Colitis Foundation of America, the annual economic cost of IBD was $1.8 to 2.6 billion (http://www.ccfa.org).

Ethnic variation of UC and CD, with the Ashkenazi Jewish group having the highest risk, familial aggregation of UC and CD, and importantly a much greater concordance rate for monozygotic (MZ) cotwins, as compared with that for dizygotic (DZ) cotwins, all suggest that genetic susceptibility contributes to the development of the disease (for a review see Yang and Rotter[2]). Furthermore, the pattern of familial aggregation is such that UC and CD are genetically complex traits. Thus, no single gene can explain the genetic susceptibility to either disorder, and nongenetic factors may play a role in the pathogenesis of the disease as well.

Although UC and CD do occur in the same families with a higher frequency than expected by chance alone, UC and CD have many distinct clinical and subclinical features, as well as distinct genetic determinants. Clinically, inflammation in CD may occur anywhere in the gastrointestinal tract, though it occurs most frequently in the small and large intestines, whereas UC is characterized by chronic inflammation of the colonic and rectal mucosa. In addition, inflammation in CD is focally involved with skipped normal segments of the intestine, and this inflammation can affect all layers of the bowel wall (transmural in nature); in contrast, in UC, the inflammation is continuous and superficial (only the mucosa is involved). Subclinically, a subset of antineutrophil cytoplasmic antibodies with a perinuclear immunofluorescence binding pattern (pANCAs) is associated with UC but not with CD (for a review see Yang and Rotter[3]); whereas antibodies to the cell-wall mannan of *Saccharomyces cerevisiae*, termed ASCA, are associated with CD, but not UC.[4,5] Furthermore, the IBD1 locus mapped to chromosome 16 was found to be linked to CD but not to UC in most studies.[6–10] Lastly, it has been observed that most concordant affected MZ twins are either both affected with UC or with CD. All these data strongly suggest that CD and UC are, to a large extent,

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genetically distinct. Therefore, a genetic linkage study conducted in a uniform disease population, i.e., Crohn's disease alone, should have greater power to identify disease predisposing genes.

An estimated relative risk to sibs (λs) is approximately 30-40 for CD and 10-20 for UC.[11,12] The larger λs value for CD suggests the potential greater power of identifying disease susceptibility genes using a genome-wide search approach in CD families as compared with UC and other complex diseases, e.g., for Type 1 diabetes, $\lambda s = 15$[13]; for both schizophrenia and Type 2 diabetes, $\lambda s < 10$ (see reviews in King et al.[14]).

A genome-wide search on British families with multiple affected members with CD or UC identified three putative susceptibility loci on chromosomes 3, 7, and 12. The strongest evidence for linkage of both CD and UC in this study was to a chromosome 12 region spanning 41 cm (D12S368-D12S95).[15] This chromosome 12 locus linkage was also observed in a North American Caucasian family panel[16] and an European cohort,[17] but was not detected in two other North American Caucasian samples.[18,19] The aim of our study was to evaluate whether there is evidence of linkage between CD or between UC and a chromosome 12 locus in an independent set of U.S. Caucasian families (36% being of Ashkenazi Jewish origin). Our results provided further evidence that the region on chromosome 12 is likely to contain a gene predisposing to CD.

METHODS

Subjects

Patients diagnosed with CD or UC were ascertained from the IBD programs at Cedars-Sinai Medical Center (CSMC, Los Angeles, CA) or referred by the Crohn's and Colitis Foundation of America, Inc. (New York, NY). The study protocols were approved by the CSMC Human Subject Review Committee. The diagnosis of CD or UC was documented by conventional endoscopic, histopathological, and clinical criteria.[20] Families with two or more siblings affected with CD or UC were selected for the current study. The age ranges at the onset of the diseases for affected sibs were 6–47 years (mean, 21.6) for CD and 4–46 years (mean = 25.3) for UC. Thirty-five percent of CD cases and 26% of UC cases had an age of onset of 18 years or younger.

A total of 65 CD sib pairs from 46 CD only families (families with no known history of UC) and their parents and unaffected sibs were tested for linkage with microsatellite markers on chromosomes 12 (Table 1A). A panel of UC families and mixed (with both UC and CD cases) families was used to further test the regions showing significant linkage in CD families (Table 1B). Our sample population is composed of Caucasians from North America, 36% being of Ashkenazi Jewish origin.

Genotyping

Genomic DNA was isolated from Epstein–Barr virus (EBV) transformed lymphoblastoid cell lines using QIAamp Tissue columns (Qiagen Inc., Chatsworth, CA) following the manufacturers instructions. All markers are part of the ABI Prism linkage mapping panels (Perkin Elmer/Applied Biosystems Division, Foster City, CA). Genotyping for each of the selected markers was performed by PCR amplification using 75 ng of genomic DNA and 25 ng of each oligonucleotide primer in 15 ul amplification reactions consisting of 10 mM Tris-HCl pH 9.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1% Triton X-100, 0.01% (w/v) gelatin, 250 mM of each dNTP, and 0.6 U of Ampli-Taq DNA polymerase (Perkin Elmer/Applied Biosystems Division). Amplification conditions consisted of an initial denaturation at 95°C for 5 minutes, 10 cycles of denaturation at 94°C for 15 seconds, annealing at 55°C for 15 seconds, and extension at

Table 1A
Description of the family panels used for linkage analysis. CD only families: Count in each category by number of affected sibs in a family

Affected sibs	Families	CD sibs	Unaffected sibs	Parents	Individuals genotyped	Sib pairs
2	38	76	37	64	177	38
3	7	21	6	13	40	21
4	1	4	1	0	5	6
Total	46	101	44	77	222	65

Table 1B
Description of the family panels used for linkage analysis. UC and mixed families: Count in each category by number of affected sibs in a family

IBD sibs	Families	CD sibs	UC sibs	Unaffected sibs	Parents	Individuals genotyped	Sib pairs
2	25	12	38	33	42	125	25
3	3	0	9	9	6	24	9
5	1	4	1	0	2	7	10
Total	29	16	48	42	50	156	44

72°C for 30 seconds, followed by 20 cycles with parameters that were the same as the first 10 cycles, with the exception of a reduction of the denaturation temperature to 89°C for the latter. The resulting PCR products were pooled and diluted approximately 20-fold and denatured before loading on a 36 cm long, 0.2 mM thick, 4.25% (6 M Urea) denaturing polyacrylamide gel. Data were collected with an ABI 377 DNA sequencer using ABI PrismTM data Collection software (v. 1.1, PE Applied Biosystems, Foster City, CA). Initial analyses of the gels were accomplished with ABI GeneScan (v. 2.0.2) software. Allele sizes were determined with ABI Genotyper software (v. 1.1). Fluorescently labeled oligonucleotide primers were purchased from ABI.

The polymorphic markers analyzed in this study are shown in Table 2. Average distance between two neighboring markers is 11.1 cm.

Statistical analysis

Two point linkage analysis was performed using the SIBPAL subroutine program (version 2.7)[21] of the Statistical Analysis for Genetic Epidemiology (SAGE) package.[22] This computerized sib pair analysis program estimates the proportion (π) of alleles that the sib pair shares identical-by-descent at that locus. The observed mean proportion of alleles shared by sib-pairs were compared with expected 0.5 by z-test (one-sided). If the linkage between disease and locus exists, an increase of π in concordant affected should be observed. Because we were testing specific chromosomal regions reported previously, $p < 0.01$ from the two point linkage analysis was considered to be statistically significant as recommended by Lander and Kruglyak.[23]

The MAPMAKER/SIBS program was employed for multipoint linkage analysis to compute maximum likelihood values of the allele sharing proportions (z_0, z_1, z_2) at each location along the genome and then to compute a maximum LOD score Z at each location.[24] Maximum likelihood proportions were estimated under the possible triangle constraint (i.e., $z_0 + z_1 + z_2 = 1$; z_1 (1/2; z_1 ($2z_0$) and using all independent sib-pairs. Genetic distances between the markers were from published Genethon maps.[25]

Allele frequencies for each marker were estimated from parents in the sample. Jewish and non-Jewish families were tested together, as well as separately to examine for potential ethnic heterogeneity. Allele frequencies for Jewish and non-Jewish samples were estimated separately. Because we genotyped parents in most families and genotyped all available unaffected sibs (data from unaffected sibs were used to infer parental genotypes when parents were not studied), the estimated allele frequencies will have no or little effect on the linkage results.

RESULTS

Two-point linkage analysis of chromosome 12 markers

Because we have available a relatively larger number of CD only families, as compared with UC and mixed families, our initial testing was carried out in the CD only families. As shown in Table 2, we observed significant linkage at the same region as reported in British families on chromosome 12. However, the marker with our highest p value (D12S85) is approximately

Table 2
Polymorphic markers of chromosome 12 and results of two point linkage analysis in CD only families

		All families		Jewish families		Non-Jewish families	
Markers	Distance (cM)	Mean (π)	p value	Mean (π)	p value	Mean (π)	p value
D12S352	0.0	0.55	0.093	0.51	0.42	0.57	0.063
D12S99	13.8	0.54	0.20	0.52	0.38	0.55	0.20
D12S336	22.1	0.51	0.44	0.49	1.0	0.52	0.39
D12S364	31.9	0.58	0.044	0.61	0.068	0.56	0.16
D12S310	39.0	0.56	0.056	0.62	0.039	0.52	0.31
D12S345	56.3	0.59	0.017	0.64	0.027	0.56	0.13
D12S85	64.6	0.62	0.0004	0.61	0.031	0.63	0.0027
D12S368	68.8	0.58	0.026	0.67	0.0048	0.53	0.33
D12S83	77.0	0.52	0.25	0.54	0.27	0.52	0.37
D12S326	90.7	0.48	1.0	0.42	1.0	0.52	0.35
D12S351	100.0	0.47	1.0	0.41	1.0	0.51	0.40
D12S346	110.0	0.49	1.0	0.43	1.0	0.52	0.37
D12S79	130.3	0.45	1.0	0.58	0.13	0.38	1.0
D12S86	139.3	0.49	1.0	0.60	0.13	0.42	1.0
D12S324	152.8	0.49	1.0	0.51	0.46	0.49	1.0
D12S367	166.8	0.50	0.46	0.53	0.33	0.49	1.0

Table 3
Two point linkage results in UC and mixed families

Chromosome 12			
Markers	Numbers of pairs	Mean (π)	p value
D12S352	40	0.49	1.0
D12S364	38	0.48	1.0
D12S310	43	0.48	1.0
D12S345	39	0.52	0.33
D12S85	39	0.48	1.0
D12S368	40	0.44	1.0

12 cm away from the marker showing the best evidence for linkage in the British sample (D12S83, $p = 0.25$). The mean allele sharing at marker D12S85 is 0.62 ($p = 0.0004$), and is 0.59 (D12S368, $p = 0.017$) and 0.58 (D12S345, $p = 0.026$) at the two neighboring markers. In this region, the smallest observed p values in Jewish families are at D12S368 ($p = 0.0048$) and in non-Jewish families at D12S85 ($p = 0.0027$).

Multipoint linkage analysis demonstrated similar results as the two-point analysis (Fig. 1). More specifically, there was a peak of LOD score around marker D12S85 at 64 cm from the p telomere (LOD = 2.0).

Heterogeneity between CD and UC

To examine the potential role of the chromosome 12 locus in UC, we used markers that showed excess allele sharing among affected CD sib-pairs (D12S364, D12S310, D12S345, D12S85, D12S368) to genotype our UC and UC/CD mixed sib pairs from UC and mixed families. The mean values for alleles shared identical-by-decent were approximately 0.5 (± 0.02) with these markers (see Table 3). When the observed mean value of alleles shared identical-by-descent in the CD only sib pairs at D12S85 was compared with that observed in UC and mixed sib pairs, the difference between the two groups reached statistical significance at $p = 0.02$ (two-sided test). The chromosome 12 locus therefore appears to contribute specifically to CD in our population.

DISCUSSION

In our North American Caucasian families, we have provided further evidence to support the findings of Satsangi et al.[15] regarding the presence of a region on chromosome 12 that appears to encode a gene predisposing to CD. However, the region showing the significant linkage (D12S364 to D12S368) doesn't perfectly overlap with the region reported by Satsangi et al., (D12S368 to D12S95) and the markers with the best evidence for linkage (i.e., the smallest p value) were separated by approximately 12 cm. This difference could not be explained by the informativeness of the markers in the region because the information content for the markers in the two regions was comparable in our sample (70% ~ 80%). As demonstrated in Figure 1, Jewish and non-Jewish samples appear to have different peaks. The peak in non-Jewish sample appears to overlap the peak observed in British families, whereas the peak in the Jewish samples appears to be approximately 10 cm proximal. The possibility that there is more than one susceptibility locus on chromosome 12 can not be ruled out. However, additional markers in the region and additional families are needed to delineate the precise region con-

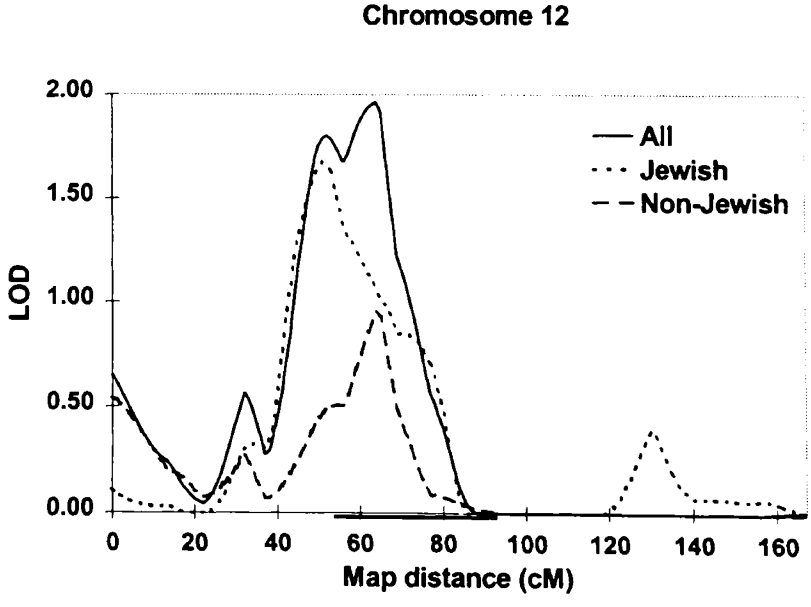

Fig. 1 Maximum LOD score at each location along the chromosome for chromosome 12 for all CD only families as a whole and for Jewish and non-Jewish families separately. The best LOD score (2.0) is located at 64 cm on chromosome 12. The highlighted areas on the map distance axes are the approximate regions demonstrating positive linkage with IBD in the previously reported British study.[15]

taining the CD susceptibility loci.

Unlike Satsangi et al.,[15] Duerr et al.,[16] and Curran et al.,[17] in which the significant evidence of linkage with the chromosome 12 region was reported in both UC and CD sib pairs together as well as separately, we did not observe any linkage between the region on chromosome 12 with UC in our UC and mixed families. This could be possibly due to the lower power in our UC and mixed families because of the smaller number of families. However, there was no trend toward distortions because the sib pair sharing in these latter families ranged from 0.44 to 0.52, which was very close to the random expectation of 0.5. Given other negative reports for linkage in this region,[18,19] such discrepancy may be evidence for disease heterogeneity or population variation.

It is likely that pooling data and stratifying by ethnic groups will aid in identifying susceptibility genes for any genetically complex diseases. However, until they are available, it is important to report even modest linkage results and review them as a whole, rather than focus on a single *p* value or LOD score. As of today, both IBD1 on chromosome 16 and a locus on chromosome 12 have been observed to be linked with either CD only or CD and UC by multiple IBD genetic research groups from Europe and North America.[6,7,9,10,15–17,26,27] Thus for this complex disease, there is substantial evidence for the involvement of at least two loci.

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