The IBD International Genetics Consortium Provides Further Evidence for Linkage to IBD4 and Shows Gene–Environment Interaction

Marie Pierik, MD, Huiying Yang, MD, PhD,† Michael M. Barmada, MD, PhD,‡ Juleen A. Cavanaugh, MD, PhD,§ Vito Annese, MD, PhD,‖ Steven R. Brant, PhD,¶ Judy H. Cho, PhD,** Richard H. Duerr, PhD,†† Jean-Pierre Hugot, MD, PhD,‡‡ Dermot P. McGovern, PhD,§§ Paulina Paavola-Sakki, MD, PhD,‖‖ Graham L. Radford-Smith, PhD,¶¶ P. Pavli, PhD,*** Mark S. Silverberg, PhD,††† Stephan Schreiber, MD, PhD,‡‡‡ Kent D. Taylor, PhD,† and Robert Vlietinck, PhD,§§§ for the IBD International Genetics Consortium*

Background and Aims: The inflammatory bowel diseases (IBDs) Crohn's disease (CD) and ulcerative colitis are complex disorders with an important genetic determinant. One gene associated with CD has been identified: *NOD2/CARD15*. Two independent genome-wide scans found significant evidence (logarithm of odds [LOD] 3.6) and suggestive evidence (LOD 2.8) for linkage on locus 14q11-12, also known as the IBD4 locus. To further characterize this locus, we assessed gene–environment interaction (IBD4 × smoking) and phenotypic heterogeneity in a large cohort of IBD-affected sibling pairs as part of an ongoing international collaborative effort.

Patients and Methods: A total of 733 IBD families, comprising 892 affected sibling pairs, were genotyped for microsatellites D14S261, D14S283, D14S972, and D14S275, spanning the IBD4 locus. Information on gender, ethnicity, age at onset, smoking at diagnosis, extraintestinal manifestations, and disease location was available.

Results: A significant distortion in the mean allele sharing (MAS) between affected siblings was observed for CD patients only at each of the four markers (54.6%, 52.8%, 50.4%, and 53.3%, respectively). Maximum linkage for CD was observed at marker D14S261 (multipoint nonparametric linkage score 2.36; $P \leq 0.01$; MAS 54.6%). MAS was higher in CD families in which all siblings or at least one sibling smoked compared with nonsmoking CD families (MAS, 58.90%, 57.50%, and 52.80%, respectively).

Conclusions: The IBD International Genetics Consortium replicated the IBD4 locus on chromosome 14q for CD and also showed evidence for a gene–environment interaction at this locus. Further studies are needed to explore the mechanism by which smoking influences IBD4.

Key Words: chromosome 14, Crohn's disease, gene–environment interaction, inflammatory bowel disease, smoking

(*Inflamm Bowel Dis* 2005;11:1–7)

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract with a prevalence of 200 to 300/100,000 in developed countries.[1] IBD is a complex disease. The two main phenotypic subgroups, Crohn's disease (CD) and ulcerative colitis (UC), usually can be distinguished by clinical, endoscopic, and histologic parameters.[2,3] The etiology of the disease remains unknown, but it is clear that both environmental and genetic factors play a role in the develop-

Received for publication August 25, 2004; accepted August 26, 2004.

From the *Department of Gastroenterology, University Hospital Gasthuisberg, Leuven, Belgium; the †Division of Medical Genetics and IBD Center, Cedar-Sinai Medical Center, Los Angeles, CA; the ‡Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; the §Medical Genetics Research Unit, Australia National University Medical School, The Canberra Hospital, Canberra, Australia; the ‖Unit of Gastroenterology, CSS-IRCCS Hospital, S. Giovanni Rotondo, on behalf of the Italian group of IBD, Italy; ¶Harvey M. and Lyn P. Meyerhoff, IBD Center, Department of Medicine, The Johns Hopkins University School of Medicine and Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland. **The Martin Boyer Genetics Research Laboratories, Gastroenterology section, Department of Medicine, The University of Chicago Hospitals, Chicago, IL; the ††Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA; ‡‡Fondation Jean Dausset/CEPH, Paris, France; the §§Gastroenterology Unit, Radcliffe Infirmary, Oxford, UK; the ‖‖Departments of Medicine and Gastroenterology, University of Helsinki, Helsinki, Finland; ¶¶Inflammatory Bowel Disease Laboratory, Royal Brisbane Hospital Foundation Clinical Research Centre, Brisbane, Australia; ***Gastroenterology Unit, The Canberra Hospital, Canberra, Australia; the †††University of Toronto, Mount Sinai Hospital, Toronto, Canada; ‡‡‡Mucosa Research Group, Christian- Albrechts University, Department of General Internal Medicine, Kiel, Germany; and the §§§Department of Epidemiology and Human Genetics, Katholieke Universiteit Leuven, Leuven, Belgium.

This study was supported by a grant from the Crohn's and Colitis Foundation of America (CCFA) for database management.

Reprints: Marie Pierik MD, UZ Gasthuisberg, Department of Gastroenterology, E 462, Herestraat 49, B-3000 Leuven, Belgium (e-mail: Marieke.Pierik@uz.kuleuven.ac.be).

ment of IBD. Nine published genome-wide scans have identified several loci of linkage.[4–12] So far, 7 chromosomal loci have been replicated in independent studies and are referred to as IBD1 to IBD7. Some loci are specific for CD (i.e., IBD1, IBD4, and IBD5) or UC (i.e., IBD2), and other loci are susceptibility loci for IBD overall (i.e., IBD3 and IBD6), reflecting the genetic heterogeneity within IBD.

The IBD1 locus on chromosome 16q was the first locus to be convincingly replicated through international collaboration by the IBD International Genetics Consortium.[13] The subsequent identification of *CARD15*, as the first gene associated with CD, occurred in 2001.[14–16] The characterization of the *IBD1* gene and the alleles that are associated with the disease highlighted the importance of the interaction between the bacterial flora of the gut and the immune system in the pathogenesis of IBD.

Chromosome 14 is an acrocentric chromosome with at least two regions of prime importance to the immune system, namely, the immunoglobulin heavy-chain region close to the telomere and the centromeric α/δ T-cell receptor region. Ma et al[7] showed suggestive evidence for linkage on chromosome 14q11.2 in their genome-wide scan of 20 Jewish and 26 non-Jewish, North American, White CD families. The maximum logarithm of odds (LOD) score was observed around marker D14S261 (maximum LOD, 2.8; $P = 0.0002$). By analyzing Jewish and non-Jewish families separately, they found that the observed linkage was largely due to the contribution of the non-Jewish families. The linkage was replicated by Duerr et al[5] in a 5-centimorgan (cM) density genome scan of 62 White CD families (8 Jewish families). A significant maximum multipoint LOD score of 3.60 was observed around the same marker, D14S261, at locus 14q11-12.[5] This locus, referred to as IBD4, was also replicated by Cho et al,[6] who demonstrated nominal evidence for linkage to the region only in the subset of mixed IBD families (both UC and CD present) in their genome-wide scan (LOD, 1.53; $P = 0.04$). The IBD4 locus spans 28 cM and contains several functional candidate genes for IBD, including the T-cell receptor α and δ genes, interleukin 17E, and a number of genes involved in apoptosis.

Failure to replicate valid linkages in complex disease analyses occur because of a number of factors. The most important among these are power limitations resulting from low sample size and genetic heterogeneity. We therefore conducted a linkage study as an ongoing international collaborative effort to replicate the IBD4 locus in CD families and to examine the linkage in UC families and mixed IBD families by genotyping a large sample of White nuclear families containing IBD-affected sibling pairs. Furthermore, gene–gene and gene–environment interactions may contribute to the observed heterogeneity. As smoking is the most consistently identified environmental risk factor for the development and severity of CD, we examined its effect on the risk of IBD at this locus.[17]

METHODS

Patients

Combining IBD families through international collaboration among 13 centers from Europe, North America, and Australia resulted in a total of 733 IBD families, comprising 892 affected sibling pairs (Table 1). Each family consisted of at least two affected siblings and both parents. Families with both parents affected were excluded, and all families were White. All centers used accepted criteria for the diagnosis of CD and UC.[2,3] In CD families, all affected siblings had CD, in UC families all siblings had UC, and the mixed families contained individuals with UC and individuals with CD. All subjects gave written informed consent, and the studies were approved by the local ethics committee or institutional review board of each center. The clinical charts of the cases were reviewed for gender, age at onset, smoking behavior at diagnosis, extraintestinal manifestations, and disease location (Table 2). Jewish ethnicity was determined by the identification of 2 or more Jewish grandparents. In smoking families, all of the affected siblings actively smoked at diagnosis. Smoking was defined as the consumption of at least 1 g of tobacco (i.e., 1 cigarette) per day. In nonsmoking families, none of the affected siblings smoked at diagnosis, and in mixed smoking families at least 1 sibling smoked at diagnosis. If 1 of the affected children had 1 or more extraintestinal disease manifestations (e.g., primary sclerosing cholangitis, arthritis, uveitis, episcleritis, conjunctivitis, mouth ulcers, pyoderma gangrenosum, or erythema nodosum), the family was classified as having extraintestinal manifestations. In families with small bowel involvement, all affected siblings had CD restricted to the small bowel. In families with colonic disease, all affected siblings had only colonic disease. Families in which CD had been diagnosed in at least 1 of the affected siblings before age 22 years were defined as early-onset families.[18]

Genotyping

Each center genotyped their families and 1 or more of 4 CEPH samples as a common standard, for 4 microsatellite markers on chromosome 14q spanning the IBD4 locus (i.e., markers D14S261, D14S283, D14S972, and D14S275) (Table 3). The sex-averaged spacing between markers was 10.37, 3.85, and 3.66 cM, respectively, according to the Decode map. The physical distance between the first and last markers was 5,857,269 Mb. The order of the markers was the same in the Marshfield, Decode, and Genethon maps. The information that can be obtained by a genetic marker is reflected by its heterozygosity.

Statistics

The data were made anonymous before they were submitted to and analyzed by the consortium. Mendelian inconsistencies in the pedigree data were identified using the Ped-

TABLE 1. Centers Involved in the Consortium With Number of Studied Families and Sibling Pairs

	Families				Sibling Pairs			
Center	**Total**	**CD**	**UC**	**Mixed**	**Total**	**CD**	**UC**	**Mixed**
Australia								
Canberra	53	38	4	11	65	48	4	13
Queensland	45	19	10	16	45	19	10	16
Canada	49	31	11	7	53	33	11	9
Europe								
Belgium	63	51	4	8	82	64	6	12
Finland	51	12	24	15	66	14	28	24
France	50	35	10	5	63	45	11	7
Germany	52	28	8	16	62	32	8	22
Italy	50	19	21	10	56	23	23	10
UK/Oxford	99	51	27	21	109	57	27	25
United States								
Baltimore	50	36	5	9	65	49	5	11
Chicago	49	32	4	13	60	43	4	13
Los Angeles	59	40	6	13	71	52	6	13
Pittsburgh	63	40	10	13	95	68	10	17
Total	733	432	144	157	892	547	153	192

Check program.[19] Unlikely genotypes (i.e., those causing unlikely double recombinants) were identified and removed using the Merlin program.[20]

Linkage analysis calculates the tendency of DNA sequences to be inherited together as a consequence of their physical proximity on a single chromosome. Because the precise genetic model (i.e., mode of inheritance [dominant or recessive], penetrance, and gene frequencies) for IBD is not known, model-free or nonparametric methods of linkage analysis are used. In sibling pair analyses, the number of identical alleles shared by descent (i.e., an allele inherited from the same common ancestor) is measured in pairs of affected siblings. Picking a chromosomal region at random, pairs of siblings are expected to share 0, 1, or 2 parental haplotypes with a frequency of one quarter, one half, and one quarter, respectively. Mean allele sharing (MAS) tries to show that affected siblings inherit the same chromosomal regions more often than is expected to occur by chance. The results of linkage analysis can be expressed as LOD scores, nonparametric linkage (NPL) scores, or as a genome-wide *P* value. According to the criteria of Lander and Kruglyak,[21] suggestive and significant linkage are defined as an LOD score or *P* value that would be expected to occur once or 0.05 times, respectively, by chance in a whole genome scan.

For families with more than 2 siblings, all possible pairs were formed and included in the analyses. Single and multipoint NPL analyses were performed using Aspex (version 2.5),[22] Merlin, and Genehunter 2.1.[23] The results were consistent across statistical programs, and all single and multipoint NPL scores presented in the paper were generated by Genehunter 2.1 (Table 4). The MAS was calculated with Aspex.

TABLE 2. Clinical Characteristics of Affected Siblings From CD Families (All Centers Combined)

Gender	
Male	359 (39.2%)
Female	556 (60.8%)
Ethnicity	
Jewish	109 (12.7%)
Non-Jewish	749 (87.3%)
Age at diagnosis (yr)	21.7 (17.0–27.2)*
Location of disease	
Small bowel only	242 (33.6%)
Small bowel and colon	376 (52.2%)
Colon only	102 (14.2%)
Active smoker at diagnosis	
Yes	263 (36.2%)
No	464 (63.8%)
Extraintestinal manifestations	
Yes	245 (59.8%)
No	165 (40.2%)

*Values given as median (interquartile range).

TABLE 3. Markers, Genetic Distances, and Heterozygosity

Marker	Genetic Distance (cM)		Physical Location (Mb)	Marker Heterozygosity (%)
	Sex-Averaged Marshfield	Sex-Averaged Decode		
D14S261	6.46	4.33	17,311,649	67.7
D14S283	13.89	14.7	19,160,006	85.7
D14S972	21.51	18.55	20,820,721	96.4
D14S275	28.01	22.21	23,168,918	67.7

A permutation test was used to calculate empirical significance (*P* value) for the comparison of observed NPL scores between different subgroups.

RESULTS

Genotyping data were available for 97.2%, 96.2%, 97.1%, and 96.8%, respectively, of all subjects for markers D14S261, D14S283, D14S972, and D14S275. The genotypes of the founders were in Hardy-Weinberg equilibrium for all markers.

Single and multipoint linkage analyses of the entire data set did not show linkage or distortion of MAS for IBD overall. Similarly, no linkage was observed for UC and mixed IBD families (Fig. 1 and Table 5).

However, a significant distortion in the MAS was observed for families with CD only for markers D14S261, D14S283, D14S972, and D14S275 (54.6%, 52.8%, 50.4%, and 53.3%, respectively). The maximum linkage for CD was observed at marker D14S261 (multipoint NPL 2.36; $P \leq 0.01$) (Fig. 1). Some of the Los Angeles and Pittsburgh families were included in the two previously published genome-wide scans demonstrating linkage to IBD4. After excluding all of the Los Angeles families (n = 50) and Pittsburgh families (n = 50) from the analyses, we still found nominal evidence for linkage for CD (maximum multipoint NPL, 1.76; $P = 0.04$).

We subsequently sought gene–environment interactions in CD families and found evidence for an interaction between IBD4 and smoking. That is, linkage was only observed in CD families where at least 1 sibling smoked (mixed smoking families [n = 101]; maximum multipoint NPL, 2.63; $P < 0.01$; maximum MAS, 58.9%) and in families in which all siblings smoked (n = 67) (maximum multipoint NPL, 1.93; $P = 0.02$; maximum MAS, 57.5%), and not in nonsmoking CD families (n = 155) (maximum multipoint NPL, 0.67; $P = 0.26$; maximum MAS, 52.8%) (Fig. 2 and Table 5). A permutation test showed that the observed difference in NPL scores of 1.96 between smoking families (NPL, 2.63) and nonsmoking families (NPL, 0.67) was only observed 65/10,000 times when random groups of the same size were selected ($P = 0.0065$).

In addition, linkage analyses conditioned on phenotypic subgroups were performed for the CD families. A maximum multipoint NPL for the Jewish CD families (n = 52) was 1.72 around marker D14S283, and for the Non-Jewish CD families (n = 352) it was 1.85 around marker D14S261.

The MAS at marker D14S261 was higher in early-onset CD families compared with late-onset CD families (0.54 and 0.51, respectively). We found 58 families in which all siblings had CD limited to the small bowel, 261 families in which both ileal and colonic disease were present, and 19 families in which all siblings had colonic CD only. The maximum multipoint NPL scores for these subgroups were 1.52, 1.46, and –1.54, respectively, and the MAS at marker D14S261 was 55.1%, 54.0%, and 37.5%, respectively (Table 5). It was remarkable that the subgroup of patients with colonic CD

TABLE 4. Single-point NPL Result for Each Marker

Marker	NPL						*P* Value					
	Genehunter 2.1			Merlin			Genehunter 2.1			Merlin		
	CD	UC	Mixed	CD	UC	Mixed	CD	UC	Mixed	CD	UC	Mixed
D14S261	1.68	−0.52	−0.42	1.60	−0.06	−0.06	0.04	0.67	0.67	0.01	0.7	0.7
D14S283	1.52	−0.77	−0.03	1.00	−0.33	−0.03	0.06	0.74	0.51	0.02	0.9	0.6
D14S972	0.22	−1.18	0.05	0.46	−0.71	−0.02	0.40	0.65	0.48	0.07	1.0	0.6
D14S275	1.79	−0.73	0.83	0.98	−0.26	0.06	0.03	0.64	0.20	0.02	0.9	0.3

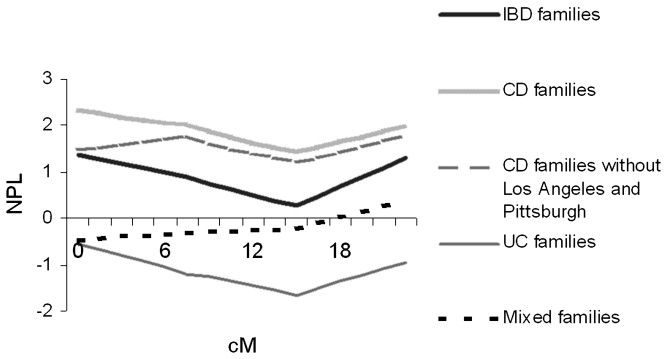

FIGURE 1. Multipoint NPL results for the region studied on chromosome 14.

showed a negative NPL score (−1.54) that is similar to the low score in the UC families (multipoint NPL, −0.54). To further examine whether the linkage to IBD4 is more associated with ileal disease than with colonic disease, we reanalyzed all CD and mixed families (n = 404) with ileal involvement (pure ileal plus ileocolonic involvement), and compared these to the families with pure colonic involvement (colonic only CD plus mixed + UC families [n = 186]). The NPL scores in both groups were 1.09 and –1.39, respectively (Fig. 3). There was no interaction between smoking and ileal disease in CD families.

Finally, in families in which at least 1 of the siblings had extraintestinal disease manifestations (n = 184), the maximum multipoint NPL score was 0.85, compared with 1.92 in families without extraintestinal manifestations (n = 84).

The *CARD15/NOD2* genotypes were known for all subjects: 25.5% of the CD patients were heterozygous and 21.1% were compound heterozygous or homozygous for the 3 main *CARD15* variants, Arg702Trp, Gly908Arg, and Leu1007fsinsC; and 14.7% of the UC patients carried 1 or more *CARD15* mutations. The maximum NPL score in CD families without *CARD15* variants (n = 204) was 1.96 and was not different from that obtained in families in which 1 or more siblings carried a *CARD15* variant (NPL, 1.92; n = 207).

DISCUSSION

Two independent genome-wide scans have previously demonstrated linkage for CD only to the pericentromeric region of chromosome 14 (14q11-12), and the region is referred to as IBD4.[5,7] In this very large cohort of nuclear IBD families, we found nominal evidence for the linkage and distortion of MAS in CD families after excluding those cohorts that initially reported linkage to this region. No linkage was observed in UC and mixed IBD families. Therefore, the IBD International Genetics Consortium was able to replicate, through international collaboration, the IBD4 susceptibility locus in a White population and show that it is specific to CD. No interaction between the known *CARD15* risk alleles and IBD4 was observed.

The expression of IBD in a given patient is the result of the interaction between the gene products of a number of susceptibility loci. Additionally, individuals are exposed to different environmental risk factors, which are determined in part by their lifestyle (e.g., non-smokers compared with smokers). Smoking is a well-established risk factor for the development of CD. A meta-analysis[17] of case-control and epidemiological studies published in 1989 showed that smokers have a 2-fold increased risk for the development of CD. CD patients who smoke have more severe disease in comparison with nonsmok-

TABLE 5. MAS (ASPEX)

	Marker			
Variable	**D14S261**	**D14S289**	**D14S972**	**D14S275**
IBD families	51.8	50.8	49.2	52.1
UC families	47.9	46.8	44.6	47.2
Mixed families	47.2	48.6	49.4	52.4
CD families	54.6	52.8	50.4	53.3
CD without LA and Pittsburgh families	51.6	52.2	50.4	53.2
Smoking families	53.7	52.5	53.4	57.5
Mixed smoking families	58.9	58.8	54.5	53.8
Nonsmoking families	52.8	47.2	45.3	48.9
Ileal disease	55.1	52.4	56.6	60.3
Ileal and colonic disease	54.0	51.8	49.0	50.6
Colonic disease	37.5	48.3	52.0	42.4
Early onset	0.54	0.53	0.50	0.51
Late onset	0.51	0.52	0.51	0.55

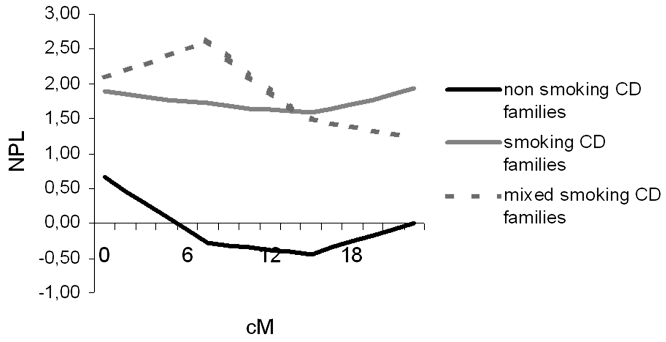

FIGURE 2. Multipoint NPL results for smoking CD families (all siblings actively smoking at diagnosis), nonsmoking IBD families (none of the siblings smoking at diagnosis), and mixed smoking IBD families (smoking and nonsmoking siblings at diagnosis).

ing patients. Smokers also have a higher relapse rate of the disease after surgery.[24] In contrast, UC has been shown to be inversely related to tobacco smoking. Interestingly, a recent study showed that in mixed IBD families, smoking siblings tended to have CD and nonsmoking siblings tended to have UC.[25] In the mixed families of the current data set, 37.5% of the CD patients smoked compared with only 22.5% of the UC patients ($P = 0.01$), and in the 32 sibling pairs discordant for smoking we found that 20 CD patients and only 12 UC patients were smokers.

This large cohort of CD families was an excellent study population in which to assess gene–environment interaction (i.e., IBD4 × smoking). Gene–environment interaction implies that, in combination, the risk associated with a genotype taken with the risk associated with an environmental factor is greater than the additive effects of each independently. Although many complex diseases may be the result of gene–environment interactions, this has been demonstrated for only a few disorders. In patients with coronary heart disease, both environmental factors (e.g., diet, exercise, and smoking) and genetic components (i.e., polymorphisms in the lipoprotein lipase gene) contribute to its pathogenesis. In a large prospective study of 2700 healthy men, a clear gene–environment interaction between the lipoprotein lipase-Asp9Asn variant and smoking was demonstrated.[26]

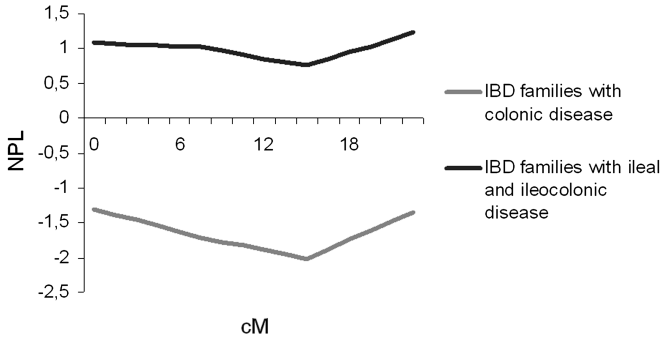

FIGURE 3. Multipoint NPL results for families with only colonic disease and families with ileal or ileocolonic disease.

In the present study, we found evidence for an interaction between smoking and the IBD4 locus, that is, only families in which at least 1 of the affected siblings was an active smoker at the time CD was diagnosed showed linkage to IBD4. The exact mechanism by which smoking influences the IBD4 locus is unknown. Most likely, the IBD4 locus in combination with smoking selects a particular phenotypic subgroup that still needs to be defined.

One other hypothesis might be that the IBD4 locus predisposes to CD and that smoking would enhance this effect or would lead to its clinical expression. An example of this kind of interaction is provided for the aldehyde dehydrogenase gene (*ALDH2*) and alcohol consumption. A polymorphism in exon 3 in the *ALDH2* gene results in ALDH2 deficiency in 50% of the Chinese, Japanese, and Korean populations due to a diminished activity and an increased turnover of the protein. Subjects with the ALDH2*2 allele respond differently to alcohol intake, and experience flushing, nausea, and tachycardia due to a rise in acetaldehyde levels.[27] Similarly, our results might be caused by a gene that catalyzes the degradation of one of the components of tobacco.

A third explanation is that IBD4 harbors a susceptibility gene predisposing both to CD and to smoking behavior, or linkage might be due to the presence of 2 genes, 1 smoking gene and a second gene that increases the risk for CD that are in linkage disequilibrium.

We conclude that the IBD4 locus is a CD-specific IBD susceptibility locus, and that there is an interaction between IBD4 and smoking. We believe that more studies on gene–environment interaction in IBD are needed and that these will not only improve our understanding of disease pathology at the molecular level but may also lead to specific advice for at-risk subjects.

ACKNOWLEDGMENTS

We wish to acknowledge the contribution of the families studied and the collaborating physicians who assisted in the collection of the samples.

REFERENCES

1. Marshall JK, Hilsden RJ. Environment and epidemiology of inflammatory bowel disease. In: Satsangi J, Sutherland LR, eds. *Inflammatory Bowel Disease*. Vol 2. New York, NY: Churchill Livingstone; 2003:17–28.
2. Travis S, Jewell DP. Ulcerative colitis: clinical presentation and diagnosis. In: Satsangi J, Sutherland LR, eds. *Inflammatory Bowel Disease*. Vol 13. New York, NY: Churchill Livingstone; 2003:169–182.
3. Forbes A. Clinical presentation and diagnosis of Crohn's disease. In: Satsangi J, Sutherland LR, eds. *Inflammatory Bowel Disease*. Vol 14. New York, NY: Churchill Livingstone; 2003:183–189.
4. Satsangi J, Parkes M, Louis E, et al. Two stage genome-wide search in

inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3,7 and 12. *Nat Genet*. 1996;14:199–202.
5. Duerr RH, Barmada M, Zhang L, et al. High-density genome wide scan in Crohn's disease shows linkage to chromosome 14q11-12. *Am J Hum Genet*. 2000;66:1857–1864.
6. Cho JH, Nicolae DL, Gold LH, et al. Identification of novel susceptibility loci for inflammatory bowel disease on chromosome 1p, 3q and 4q: evidence for epistasis between 1p and IBD1. *Genetics*. 1998;95:7502–7507.
7. Ma Y, Ohmen JD, Li Z, et al. A genome-wide search identifies potential new susceptibility loci for Crohn's disease. *Inflamm Bowel Dis*. 1999;5: 271–278.
8. Hampe J, Shaw SH, Saiz R, et al. Linkage of inflammatory bowel disease to human chromosome 6p. *Am J Hum Genet*. 1999;65:1647–1655.
9. Rioux JD, Silverberg MS, Daly MJ, et al. Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *Am J Hum Genet*. 2000;66:1863–1870.
10. Paavola-Sakki P, Ollikainen V, Helio T, et al. Genome-wide search in Finnish families with inflammatory bowel disease provides evidence for novel susceptibility loci. *Eur J Hum Genet*. 2003;11:112–120.
11. Williams CN, Kocher K, Lander ES, et al. Using a genome-wide scan and meta-analysis to identify a novel IBD locus and confirm previously identified IBD loci. *Inflamm Bowel Dis*. 2002;8:375–381.
12. Barmada MM, Brant S, Nicolae DL, et al. A genome scan in 260 inflammatory bowel disease-affected relative pairs. *Inflamm Bowel Dis*. 2004; 10:15–22.
13. The IBD International Genetics Consortium. International collaboration provides convincing linkage replication in complex disease trough analysis of a large pooled data set: Crohn's disease and chromosome 16. *Am J Hum Genet*. 2001;68:1165–1171.
14. Ogura Y, Bonen DK, Inohara N, et al. A frame shift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature*. 2001;411:603–606.
15. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature*. 2001; 411:437–439.
16. Hampe J, Cuthbert A, Croucher PJP, et al. Association between insertion mutaton in NOD2 gene and Crohn's disease in German and British populations. *Lancet*. 2001;357:1925–1928.
17. Calkins BM. A Meta-analysis of the role of smoking in inflammatory bowel disease. *Dig Dis Sci*. 1989;34:1841–1854.
18. Brant SR, Panhuysen CI, Bailey-Wilson JE, et al. Linkage heterogeneity for the IBD1 locus in Crohn's disease pedigrees by disease onset and severity. *Gastroenterology*. 2000;119:1483–1490.
19. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet*. 1998;63: 259–266.
20. Abecasis GR, Cherny SS, Cookson WO, et al. Merlin: rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet*. 2002;30:97–101.
21. Lander ES. Kruglyak Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet*. 1995;11:241–247.
22. Hinds DA, Risch N. The ASPEX package: affected sib-pair exclusion mapping. Available at: http://aspex.sourceforge.net/. Accessed 1996.
23. Kruglyak L, Daly MJ, Reeve-Daly MP, et al. Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet*. 1996;58:1347–1363.
24. Cottone M, Rosselli M, Orlando A, et al. Smoking habits and recurrence in Crohn's disease. *Gastroenterology*. 1994;106:643–648.
25. Bridger S, Lee JC, Bjarnason I, et al. In siblings with similar genetic susceptibility for inflammatory bowel disease, smokers tend to develop Crohn's disease and non-smokers develop ulcerative colitis. *Gut*. 2002; 51:21–25.
26. Talmud PJ, Bujac S, Hall S, et al. Substitution of asparagine for aspartic acid at residue 9 (D9N) of lipoprotein lipase markedly augments risk of coronary heart disease in male smokers. *Atherosclerosis*. 2000;149: 75–81.
27. Takeshita T, Yang X, Morimoto K. Association of the ADH2 genotypes with skin responses after ethanol exposure in Japanese male university students. *Alcohol Clin Exp Res*. 2001;25:1264–1269.

APPENDIX

The IBD International Genetics Consortium

Members of the consortium are listed according to the contributing center with which they collaborated.

Australia: (Canberra) J. A. Cavanaugh, P. Pavli, H. Rodgers, and N. Risch (Stanford University); (Brisbane) R. Eri, T. Florin, E. Fowler, and G. L. Radford-Smith.

Canada: M. Daly, J. Rioux, M. S. Silverberg, and H. Steinhart.

Europe: (Belgium) M. Pierik, P. Rutgeerts, S. Vermeire, and R. Vlietinck; (France—European Consortium of the Genetics of IBD) J.-P. Hugot, S. Lesage, and H. Zouali; (Finland) T. Helio, K. Kontula, P. Paavola-Sakki, and U. Turunen; (Germany) P. J. P. Croucher, J. Hampe, S. Nikolaus, and S. Schreiber; (Italy) V. Annese, A. Latiano, G. Lombardi (CSS-IRCCS Hospital), and F. Castiglione (University of Naples); and (United Kingdom, Oxford) D. Jewell, N. Lench, D. McGovern, and D. Van Heel.

United States: (Baltimore) S. Brant, J. E. Bailey-Wilson (National Human Genome Research Institute), A. S. Karban, and C. I. M. Panhuysen (Boston University); (Chicago) J. H. Cho, T. Moran, D. L. Nicolae, and E. Swanson; (Los Angeles) Y.-C. Lin, J. I. Rotter, K. D. Taylor, and H. Yang; (Pittsburgh) J.-P. Achkar, M. M. Barmada, R. H. Duerr, and L. Zhang.