Am. J. Hum. Genet. 73:1465–1466, 2003

Further Evidence of *IBD5/CARD15 (NOD2)* Epistasis in the Susceptibility to Ulcerative Colitis

To the Editor:

We read with interest the two recent articles describing analyses of the *IBD5* (MIM 606348) risk haplotype and inflammatory bowel disease (IBD) in European cohorts (Giallourakis et al. 2003; Mirza et al. 2003). In both European cohorts, the association with the *IBD5* risk haplotype and Crohn disease (CD [MIM 266600]) was replicated. Mirza et al. (2003) additionally provided evidence for interaction between *IBD5* and *CARD15 (NOD2)* (MIM 605956) in CD, a finding not seen in the subsequent paper by Giallourakis et al. (2003) or in our own transmission/disequilibrium testing (TDT) an alysis (Negoro et al. 2003). Subsequent genotype-phenotype analysis by Giallourakis et al. (2003) found no association between *IBD5* and clinical subgroups of patients with CD. In contrast, our group has recently published a case-control study of U.K. whites demonstrating that the association between *IBD5* and CD was confined to those individuals with perianal disease (Armuzzi et al. 2003). It would be interesting to know whether association with this particular phenotype was evaluated in the Giallourakis cohort.

In 187 trios with ulcerative colitis (UC [MIM 191390]), Giallourakis et al. (2003) also reported a novel association between the *IBD5* risk haplotype and UC. This association was most pronounced in those individuals possessing one of the three common CD-associated *CARD15* variant alleles, suggesting an epistatic relationship between these replicated CD loci in patients with UC. In our British patients, we observed a similar linkage disequilibrium (LD) pattern across this locus. However, using both TDT (105 transmissions to 124 nontransmissions, $P = .24$) and case-control studies, we were unable to demonstrate association between the *IBD5* risk haplotype and UC (Armuzzi et al. 2003; Negoro et al. 2003). However, stratification of the *IBD5* results in UC by *CARD15* status was not performed.

Following the report by Giallourakis et al. (2003), we therefore stratified our trios to assess the transmissions of IGR2060a_1 (an *IBD5* risk haplotype–tagging SNP) from heterozygous parents to affected offspring who also carried at least one *CARD15* risk allele, revealing 14 transmissions to 12 nontransmissions ($P = .78$). The lack of association may reflect a true relationship, or it may be a type I error due to the relatively weak power of this analysis. We therefore genotyped for the three common *CARD15* variants and *IBD5* haplotype–tagging SNP to assess any epistatic association in a more powerful case-control study of 278 patients with UC (largely independent of the UC trios) and 232 healthy controls (HC). We found a novel association between the *CARD15* 702Trp variant and UC (table 1). This association was not significant in the *IBD5* wild-type homozygotes but became significant in the *IBD5* heterozygotes and even more significant in the *IBD5* "risk" homozygotes, supporting the theory of an epistatic relation between the *IBD5* locus and *CARD15* in the susceptibility to UC (table 1). However, there was no such relationship between UC and the 908Arg or

Table 1

Case-Control Analysis of *CARD15* 702Trp Allele Association with UC, Stratified by *IBD5* Status

Patient Group	702Trp Allele Frequency (%)	*P* Value (compared to HC)[a]	Relative Risk (95% CIs)[a]	PAR[b]
Healthy controls (232)	2.80			
Ulcerative colitis, overall (278)	5.94	.016	2.14 (1.11–4.12)	.03
IBD5 "nonrisk" homozygotes (75)	3.33	.737	NA	NA
IBD5 heterozygotes (152)	6.25	.019	2.28 (1.11–4.70)	.04
IBD5 "risk" homozygotes (51)	8.82	.004	3.40 (1.41–8.18)	.06

[a] *P* value and RR were calculated using the Fisher's Exact Test.
[b] PAR = population attributable risk.

Table 2

Transmissions versus Nontransmissions in Common CD-Associated *CARD15* Variants from the TDT of 244 UC Trios

Variant	Transmissions	Nontransmissions	*P* Value
702Trp	24	16	.26
908Arg	3	12	.066
Leu1007fsinsC	8	15	.22

Leu1007fsinsC *CARD15* alleles (908Arg: HC 0.65%, UC 0.73%, $P = .73$; Leu1007fsinsC: HC 2.14%, UC 0.74%, $P = .057$), despite *IBD5* stratification (data not shown). We found no particular UC phenotype (need for surgery, age at onset, disease distribution) associated with the *CARD15* 702Trp allele, with or without *IBD5* stratification.

We believe that these data confirm the presence of an *IBD5/CARD15* epistatic relationship in the susceptibility to UC (although the overall population effect is relatively small [table 1]), in contrast to the nonepistatic relationship between *IBD5/CARD15* and CD seen in both our populations and that of Giallourakis et al. (2003). Our data, however, suggest that this epistatic effect is seen exclusively with the 702Trp variant, supporting other data that imply that the 702Trp polymorphism may possess unique properties not shared with the other *CARD15* CD-associated variants (Bonen et al. 2003; Rahman et al. 2003; Sugimura et al. 2003). Indeed, our data suggest a trend toward undertransmission/reduced allele frequency of 908Arg/Leu1007fsinsC in UC (table 2 and the paragraph above). Further work is needed to determine whether the epistatic *IBD5/CARD15* interaction in UC is a "global" *CARD15* phenomenon or, as we have suggested, is restricted to the 702Trp allele. These data support the theory that UC and CD are related polygenic conditions that share some, but not all, susceptibility genes.

Acknowledgments

This work was supported by the British Medical Research Council (LINK Grant, D.Mc.G. and D.v.H.), the Digestive Disorders Foundation/Belmont Trust (D.Mc.G.), the International Organization for the Study of Inflammatory Bowel Disease (D.Mc.G.), the National Association for Colitis and Crohn's Disease (T.A.), the Broad Foundation (T.A.), the Hamanako Symposium (K.N.), and Oxagen Ltd.

DERMOT P. B. MCGOVERN,[1,2] DAVID A. VAN HEEL,[1] KENICHI NEGORO,[1] TARIQ AHMAD,[2] AND DEREK P. JEWELL[2]

[1]*The Wellcome Trust Centre for Human Genetics and* [2]*Gastroenterology Department, University of Oxford, Oxford*

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for Crohn disease, ulcerative colitis, *IBD5,* and *CARD15 [NOD2]*)

References

Armuzzi A, Ahmad T, Ling KL, De Silva A, Cullen S, Van Heel D, Orchard TR, Welsh KI, Marshall SE, Jewell DP (2003) Genotype-phenotype analysis of the Crohn's disease susceptibility haplotype on chromosome 5q31. Gut 52: 1133–1139

Bonen DK, Ogura Y, Nicolae DL, Inohara N, Saab L, Tanabe T, Chen FF, Foster SJ, Duerr RH, Brant SR, Cho JH, Nunez G (2003) Crohn's disease-associated *NOD2* variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. Gastroenterology 124:140–146

Giallourakis C, Stoll M, Miller K, Hampe J, Lander ES, Daly MJ, Schreiber S, Rioux JD (2003) *IBD5* is a general risk factor for inflammatory bowel disease: replication of association with Crohn disease and identification of a novel association with ulcerative colitis. Am J Hum Genet 73:205–211

Mirza MM, Fisher SA, King K, Cuthbert AP, Hampe J, Sanderson J, Mansfield J, Donaldson P, Macpherson AJ, Forbes A, Schreiber S, Lewis CM, Mathew CG (2003) Genetic evidence for interaction of the 5q31 cytokine locus and the *CARD15* gene in Crohn disease. Am J Hum Genet 72:1018–1022

Negoro K, McGovern DP, Kinouchi Y, Takahashi S, Lench NJ, Shimosegawa T, Carey A, Cardon LR, Jewell DP, van Heel DA (2003) Analysis of the *IBD5* locus and potential gene-gene interactions in Crohn's disease. Gut 52:541–546

Rahman P, Bartlett S, Siannis F, Pellett FJ, Farewell VT, Peddle L, Schentag CT, Alderdice CA, Hamilton S, Khraishi M, Tobin Y, Hefferton D, Gladman DD (2003) *CARD15*: a pleiotropic autoimmune gene that confers susceptibility to psoriatic arthritis. Am J Hum Genet 73:677–681

Sugimura K, Taylor KD, Lin YC, Hang T, Wang D, Tang YM, Fischel-Ghodsian N, Targan SR, Rotter JI, Yang H (2003) A novel *NOD2/CARD15* haplotype conferring risk for Crohn disease in Ashkenazi Jews. Am J Hum Genet 72:509–518

Address for correspondence and reprints: Dr. Dermot P. B. McGovern, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Headington, Oxford, OX3 7BN, United Kingdom. E-mail: dermot@well.ox.ac.uk

0002-9297/2003/7306-0024$15.00