PostScript

LETTERS

IBD5 is associated with an extensive complicated Crohn's disease feature: implications from genotype–phenotype analysis

Currently, decisions regarding patient care in Crohn's disease are largely dictated by clinical phenotypes, incorporating disease localisation and behaviour. In this context, a molecular classification based on genetic susceptibility can provide a far more meaningful stratification for biologically relevant genotype–phenotype associations, and ultimately, the individualisation of patient treatment.¹

The IBD5 risk haplotype (IBD5^{risk}) located within the 5q31 cytokine gene cluster has been unequivocally associated with Crohn's disease.² Specific clinical phenotypes have not been firmly established for IBD5^{risk}, although an association with perianal and with fistulising Crohn's disease has been proposed.^{3–8} We performed genotype–phenotype correlations for IBD5^{risk} and Crohn's disease in a cohort of 325 German patients, described elsewhere,³ to determine whether IBD5^{risk} is associated with specific localisation phenotypic features and, in particular, with an extensive disease feature. Disease characteristics and age of onset were also analysed. Phenotyping procedures have been tested for interobserver and intraobserver stability, and quality criteria for phenotype information have been developed and applied. The IBD5^{risk} was defined using the risk allele at SNP IGR298a_1 as a proxy, given its central location within this region. We adopted two different approaches to fully explore disease localisation data for IBD5 (ie specific site of involvement and extent of involvement): (1) the classical approach of studying ileal Crohn's disease, colonic Crohn's disease and perianal Crohn's disease regardless of the presence of inflammation in the other two sites; (2) entailing the construction of mutually exclusive categories that allow us to focus on the number of sites (and which ones) in which the disease is present. We compared IBD5^{risk} frequencies using the Pearson's χ^2 test. Table 1 shows the results of the test.

From the first approach, we did not find any difference in the percentage of patients with IBD5^{risk} in the three sites. From the mutually exclusive categories, it emerges that, even with smaller numbers, patients with at least one risk allele (IBD5^{risk}) are more frequent in categories including the ileal site with something else (especially categories 4 and 7).

Given the results in table 1, we looked for an association of IBD5^{risk} with the "ileal+other" category using a logistic regression model adjusting for age of onset, smoking status, sex and CARD15 status. We found that being an IBD5^{risk} carrier almost doubles the probability of developing Crohn's disease in the ileum and somewhere else at the same time (odds ratio (OR) 1.77 (95% confidence interval (CI) 1.1 to 2.9)). No difference was found between homozygous and heterozygous status, suggesting that one copy of the risk allele is enough to confer risk of developing an "extended" Crohn's disease phenotype. No

significant association was found for the ileal only category. CARD15 and smoking status were not associated with the "ileal+other" category.

For Crohn's disease behaviour, we found a significantly lower percentage of patients with IBD5^{risk} with pure inflammatory Crohn's disease compared with those with fistulising or stricturing Crohn's disease ($p = 0.01$). In the multivariate analyses, fistulising behaviour was seen to be associated with IBD5^{risk} (OR 5.61 (95% CI 1.9 to 16.4)) and there seems to be a dose haplotype effect given that the OR for homozygosity is higher than that for heterozygosity (OR 6.5 (95% CI 1.6 to 26.5) v 4.8 (1.5 to 14.8), respectively). Stricturing behaviour was also associated with IBD5, but only in homozygous patients (OR 3.45 (95% CI 1.2 to 9.5)).

The probability of being an IBD5^{risk} carrier decreases with age of onset but the OR did not reach statistical significance (OR = 0.97 per year of age (0.94–1.00)).

In summary, adopting an alternative approach to genotype–phenotype correlations—that is, looking at mutually exclusive

categories based on the number of sites involved, we have defined an association of IBD5^{risk} with an "extensive complicated disease" feature in Crohn's disease in a rigorously phenotyped European population. These findings, if replicated in a larger independent cohort, would implicate that IBD5^{risk} could act as a modifier rather than as a true susceptibility gene, leading to the development of more complex multi-site disease with co-existent ileal involvement and non-purely inflammatory behaviour, especially for patients who carry two copies of the risk allele.

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Table 1 Allele and genotype frequencies by phenotype*

Phenotypes	All			IBD5 ^{risk}	
	n	%	Allele frequency (%)	n	%
Localisation					
Ileal (1+4+5+7)	271	83.4	48.0	197	72.7
Perianal (2+4+6+7)	149	45.8	46.6	109	73.2
Colon (3+5+6+7)	160	49.2	47.5	116	72.5
				†	
One location					
1 Ileal only	101	31.1	44.1	67	66.3
2 Perianal only	10	3.1	20.0	4	40.0
3 Colon only	27	8.3	46.3	20	74.1
Two locations					
4 Ileal+perianal	54	16.6	50.9	43	79.6
5 Ileal+colon	48	14.8	49.0	34	70.8
6 Perianal+colon	17	5.2	32.4	9	52.9
Three locations					
7 Ileal+perianal+colon	68	20.9	50.7	53	77.9
				p=0.06	
Behaviour					
Inflammatory	71	21.8	38.0	44	62.0
Fistulising only	47	14.5	57.4	42	89.4
Stenotic only	63	19.4	50.8	46	73.0
Fistulising/stenotic	144	44.3	44.4	98	68.1
				p=0.01	
Sex					
Female	230	70.8	45.2	160	69.6
Male	95	29.2	48.4	70	73.7
				p=0.46	
Smoking					
Ever-smokers	167	51.4	43.4	111	66.5
Never-smokers	158	48.6	49.1	119	75.3
				p=0.08	
Age of onset					
<17	55	16.9	51.2	41	74.6
17–40	259	79.7	45.6	184	71.0
>40	11	3.4	31.8	5	45.4
				p=0.15	
Smoking					
Age at onset, mean (SD)	23.4 (7.8)			22.8 (7.5)	
All	325	100.0	46.2	230	70.8

*The p values refer to Pearson's χ^2 test comparing counts within boxes.

†These categories are not mutually exclusive and therefore were not compared.

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References

- 1 Gasche C, Grundtner P. Genotypes and phenotypes in Crohn's disease: do they help in clinical management? *Gut* 2005;54:162-7.
- 2 Rioux JD, Daly MJ, Silverberg MS, et al. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 2001;29:223-8.
- 3 Giallourakis C, Stoll M, Miller K, et al. 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* 2003;73:205-11.
- 4 Armuzzi A, Ahmad T, Ling KL, et al. Genotype-phenotype analysis of the Crohn's disease susceptibility haplotype on chromosome 5q31. *Gut* 2003;52:1133-9.
- 5 Vermeire S, Pierik M, Hlavaty T, et al. Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. *Gastroenterology* 2005;129:1845-53.
- 6 Palmieri O, Latiano A, Valvano R, et al. Variants of OCTN1-2 cation transporter genes are associated with both Crohn's disease and ulcerative colitis. *Aliment Pharmacol Ther* 2006;23:497-506.
- 7 Latiano A, Palmieri O, Valvano RM, et al. Contribution of IBD5 locus to clinical features of IBD patients. *Am J Gastroenterol* 2006;101:318-25.
- 8 Noble CL, Nimmo ER, Drummond H, et al. The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease. *Gastroenterology* 2005;129:1854-64.

Association of the macrophage migration inhibitory factor gene polymorphisms with inflammatory bowel disease

Macrophage migration inhibitory factor (MIF), an immunoregulatory cytokine that has pro-inflammatory, hormonal and enzymatic activities, has been found to be markedly increased in the serum of patients with inflammatory bowel disease (IBD).^{1,2} MIF-deficient mice failed to develop disease¹ and blockage with anti-MIF antibody reduced disease activity.^{1,2} Finally, functional polymorphisms of the human *MIF* gene have been associated with increased susceptibility to inflammatory and autoimmune diseases.³ These findings prompted us to investigate the potential association of the functional *MIF* -173G/C and -794 (CATT)_n gene variants with the susceptibility and clinical expression of IBD.

We studied a case-control cohort (cohort 1) comprising 336 patients with Crohn's disease and 287 patients with ulcerative colitis from south Spain and 361 controls from the same area. An additional cohort (cohort 2) was analysed, comprising 325 patients with Crohn's disease, 347 patients with ulcerative colitis and 526 controls from Madrid. Table 1 shows the clinical characteristics of both cohorts separately. *MIF* -173 and *MIF* CATT repeat genotyping was carried out as described previously.⁴

Evidence for association of *MIF* and IBD was found when the *MIF* -173G/C genotypes and alleles were compared between patients with IBD and controls from cohort 1 (table 2). The *MIF* -173*C allele was associated with an increased susceptibility to IBD ($p = 0.002$). Similarly, an increased -173*C allele frequency was observed in patients with IBD in cohort 2 ($p = 0.034$). In the combined analysis, the odds ratio (OR) for the estimated risk for the *MIF* -173*C allele was 1.37 (95% confidence interval 1.1 to 1.6), with an overall $p = 0.001$.

Next, we analysed the prevalence of the *MIF* -173G/C variation in patients with Crohn's disease and ulcerative colitis independently. The *MIF* -173*C allele frequency was significantly higher in patients with ulcerative colitis from cohorts 1 ($p = 0.02$) and 2 ($p = 0.01$) compared with their respective controls. Similarly, the allele frequency of the *MIF* polymorphism in patients with Crohn's disease from cohort 1 was significantly higher than in controls ($p = 0.002$). When the entire population with IBD was subdivided into ulcerative colitis and Crohn's disease cohorts, the *MIF* -173*C allele was found to be associated with both disease subgroups (Crohn's disease, pooled OR = 1.3, $p = 0.004$; ulcerative colitis, pooled OR = 1.4, $p = 0.001$). No significant differences were observed in the distribution of CATT_n genotype and allele frequencies between IBD, Crohn's disease, ulcerative colitis and controls in the two cohorts, when analysed independently or combined (data not shown). For the genotype-phenotype analysis, patients from cohorts 1 and 2 were grouped and

Table 1 Clinical features of 558 Spanish patients with Crohn's disease and 472 Spanish patients with ulcerative colitis

	Cohort 1	Cohort 2
<i>Ulcerative colitis</i>		
Sex (% M)	49	43
Mean (SD) age at onset (y)	32.3 (13.7)	37.5 (12.3)
Mean (SD) disease duration (y)	8.1 (6.4)	11.8 (7.6)
Smoking habits (%)		
Never	51.9	43.2
Ex/current	48.1	56.8
Extraintestinal manifestations		
Cutaneous (%)	41.7	46.6
Articular (%)	13.7	15.8
Ocular (%)	35.8	36.8
	3.6	3.2
Disease location (%)		
Left sided	54.8	58.6
Extensive colitis/pancolitis	45.2	41.4
Surgery (%)	15.9	12.1
Immunosuppression (%)	26	23
<i>Crohn's disease</i>		
Sex (% M)	48	47.1
Mean (SD) age at onset (y)	29.2 (11.7)	27.3 (13.4)
<40 (A1)	82.3	80.1
>40 (A2)	17.7	19.9
Mean (SD) disease duration (y)	7.9 (6.1)	11 (6.6)
Smoking habits (%)		
Never	49.2	53.9
Ex/current	50.8	46.1
Disease location		
Ileal (L1)	41.2	47.9
Colonic (L2)	16.4	16.4
Ileocolonic (L3)	38.7	32.2
Upper GI tract (L4)	3.7	3.4
Disease behaviour		
Inflammatory (B1)	36.7	43.2
Structuring (B2)	25.3	15.1
Perforating (B3)	38	41.8
Extraintestinal manifestations	37.8	36.9
Surgery (%)	43.2	41.5
Immunosuppression (%)	46.8	45.1
Infliximab (%)	12.3	10.8

GI, gastrointestinal; M, male.