aminosalicylic acid for treatment of steroid dependent ulcerative colitis. *Gut* 2006;**55**:47–53.
11 **Hyams J**, Markowitz J, Lerer T, *et al*. The natural history of corticosteroid therapy for ulcerative colitis in children. *Clin Gastroenterol Hepatol* 2006;**4**:1118–23.
12 **Decorti G**, De Iudicibus S, Stocco G, *et al*. Glucocorticoid receptor polymorphisms in inflammatory bowel disease. *Gut* 2006;**55**:1053–4.
13 **Panarelli M**, Holloway CD, Fraser R, *et al*. Glucocorticoid receptor polymorphism, skin vasoconstriction, and other metabolic intermediate phenotypes in normal human subjects. *J Clin Endocrinol Metab* 1998;**83**:1846–52.
14 **Sakaeda T**. MDR1 genotype-related pharmacokinetics: fact or fiction? *Drug Metab Pharmacokinet* 2005;**20**:391–414.

MYO9B polymorphisms in patients with inflammatory bowel disease

An abnormal function of the intestinal barrier has been found not only in patients with inflammatory bowel disease (IBD) but even in their healthy relatives, suggesting that this condition may precede disease onset by years.[1] A genetic alteration in the intestinal permeability has also been proposed to exist in patients with coeliac disease. In support of this proposal, polymorphisms in the *MYO9B* gene (the gene for myosin IXb involved in cytoskeleton remodelling) were found to be associated with increased susceptibility to coeliac disease.[2]

The *MYO9B* gene has recently been investigated in relation to IBD and produced discordant results. No association was observed in a Norwegian population,[3] but shortly afterwards an international collaboration group performed a statistically powerful study on samples collected from the UK, Netherlands, Canada and Italy in which *MYO9B* was found to be associated with IBD, and with ulcerative colitis and Crohn's disease considered separately in some populations.[4] In that study, a stronger effect was seen in ulcerative colitis than in Crohn's disease. Our aim in the present study was to evaluate the described *MYO9B* associations in a large sample of Spanish patients with IBD.

We performed a case-control study of 627 patients with ulcerative colitis and 677 with Crohn's disease recruited from three Spanish hospitals; 990 blood donors of the same ethnicity were used as controls. Written informed consent was obtained from all subjects and ethical approval for the study was obtained from the ethics committees of the hospitals. The diagnosis of ulcerative colitis and Crohn's disease was based on standard clinical, radiological, endoscopic and histological criteria. Clinical data from essentially the same cohort can be found in a previous report.[5]

Table 1 shows the distribution of *MYO9B* polymorphisms in patients with ulcerative colitis and Crohn's disease and in healthy controls. All single nucleotide polymorphisms (SNPs) conformed to Hardy-Weinberg predictions in our control sample. In all cases a strong association was seen when patients with ulcerative colitis were compared with healthy controls. However, the association with Crohn's disease was almost negligible. Moreover, when patients with ulcerative colitis were compared with those with Crohn's disease, significant differences were observed. Stratification of the patients by clinical characteristics did not show differences between the groups.

Table 2 shows the *MYO9B* haplotype distribution estimated by the expectation-maximisation algorithm. Only the most strongly associated SNPs, in tight linkage disequilibrium as shown in the previous exhaustive research in coeliac disease susceptibility, were included in our study. It is therefore not surprising that the haplotypes show results which closely mirror those found with individual polymorphisms. In particular, the rs2305767G allele is an almost perfect marker of the first haplotype (GCG) and rs1457092A allele of the second haplotype (AAA). The AAA haplotype confers a strong predisposition to ulcerative colitis compared with the GCG haplotype ($p = 0.0001$).

This study shows a clear association of *MYO9B* polymorphisms with ulcerative colitis in the Spanish population but not with Crohn's disease. This lack of association does not seem to be derived from a low statistical power (68% for rs1457092 with OR 1.20), because the size of our Crohn's disease sample was high enough to show a strong difference with ulcerative colitis. A lower association has previously been observed with Crohn's disease than with ulcerative colitis, and no association with Crohn's disease was found in the Canadian/Italian sample.[4]

The diverse results found in the previous studies are difficult to explain. It may be that the low intrinsic OR renders most of the studies underpowered and an association is found only with luck. Another explanation might be the heterogeneous nature of the disease. There is also no easy explanation for the strikingly different susceptibility to Crohn's disease and ulcerative colitis. To our knowledge, no report has claimed that there is a difference in barrier function between the two diseases. However, this is not the first time that genetic differences have been reported between these two forms of IBD.[6] Since twin concordance rate data for ulcerative colitis suggest that the heritable component is less important than for Crohn's disease, it has been proposed that environmental factors have a stronger impact in ulcerative colitis.[7] It is therefore intriguing that the *MYO9B* gene, which seems specifically to affect susceptibility to ulcerative colitis, is probably involved in loss of tolerance to environmental agents. However, functional analyses are needed to explore further the role of this gene in ulcerative colitis and other diseases.

Table 1 *MYO9B* polymorphisms in Spanish patients with ulcerative colitis, Crohn's disease and controls

	UC (n = 677) n (%)	CD (n = 627) n (%)	Controls (n = 990) n (%)	UC vs controls	CD vs controls	UC vs CD
Rs2305767						
AA	263 (39)	183 (29)	296 (30)	3*2	3*2	3*2
AG	297 (44)	318 (51)	518 (52)	p = 0.0004	p = 0.51	p = 0.001
GG	117 (17)	126 (20)	176 (18)			
A	823 (61)	684 (55)	1110 (56)	G vs A:	G vs A:	G vs A:
G	531 (39)	570 (45)	870 (44)	p = 0.007	p = 0.40	p = 0.001
				OR = 0.82	OR = 1.06	OR = 0.77
				(95% CI 0.71 to 0.95)	(95% CI 0.92 to 1.23)	(95% CI 0.66 to 0.91)
Rs1457092						
CC	258 (38)	281 (45)	403 (41)	3*2	3*2	3*2
CA	282 (42)	257 (41)	451 (46)	p = 0.002	p = 0.18	p = 0.005
AA	137 (20)	89 (14)	136 (14)			
C	798 (59)	819 (65)	1257 (63)	A vs C:	A vs C:	A vs C:
A	556 (41)	435 (35)	723 (37)	p = 0.008	p = 0.29	p = 0.0008
				OR = 1.21	OR = 0.92	OR = 1.31
				(95% CI 1.05 to 1.40)	(95% CI 0.79 to 1.07)	(95% CI 1.12 to 1.54)
Rs2305764						
GG	227 (34)	262 (42)	364 (37)	3*2	3*2	3*2
GA	300 (44)	269 (43)	469 (47)	p = 0.005	p = 0.12	p = 0.0008
AA	150 (22)	96 (15)	157 (16)			
G	754 (56)	793 (63)	1197 (60)	A vs G:	A vs G:	A vs G:
A	600 (44)	461 (37)	783 (40)	p = 0.006	p = 0.11	p = 0.00009
				OR = 1.22	OR = 0.89	OR = 1.37
				(95% CI 1.05 to 1.40)	(95% CI 0.77 to 1.03)	(95% CI 1.17 to 1.61)

Genotyping was performed by TaqMan technology under conditions recommended by the manufacturer (Applied Biosystems, Foster City, California, USA).
CD, Crohn's disease; UC, ulcerative colitis.

Table 2 *MYO9B* haplotypes in Spanish patients with ulcerative colitis, Crohn's disease and controls

Rs2305767/ rs1457092/ rs2305764	UC (n = 1356) n (%)	CD (n = 1254) n (%)	Controls (n = 1980) n (%)	UC vs controls	CD vs controls	UC vs CD
GCG	516 (38)	563 (45)	844 (43)	p = 0.008 OR = 0.83 (95% CI 0.72 to 0.95)	p = 0.20 OR = 1.10 (95% CI 0.95 to 1.27)	p = 0.0004 OR = 0.75 (95% CI 0.64 to 0.89)
AAA	549 (40)	424 (34)	713 (36)	p = 0.009 OR = 1.21 (95% CI 1.05 to 1.40)	p = 0.20 OR = 0.91 (95% CI 0.78 to 1.06)	p = 0.0004 OR = 1.33 (95% CI 1.13 to 1.57)
ACG	226 (17)	223 (18)	347 (18)	p = 0.52 OR = 0.94 (95% CI 0.78 to 1.14)	p = 0.85 OR = 1.02 (95% CI 0.84 to 1.23)	p = 0.45 OR = 0.92 (95% CI 0.75 to 1.14)
ACA	42 (3.1)	24 (1.9)	46 (2.3)	p = 0.17 OR = 1.34 (95% CI 0.86 to 2.10)	p = 0.44 OR = 0.82 (95% CI 0.48 to 1.39)	p = 0.05 OR = 1.64 (95% CI 0.96 to 2.81)
Rare haplotypes	23 (1.7)	20 (1.6)	30 (1.5)	p = 0.68 OR = 1.12 (95% CI 0.63 to 2.00)	p = 0.86 OR = 1.05 (95% CI 0.57 to 1.93)	p = 0.84 OR = 1.06 (95% CI 0.56 to 2.03)

Haplotypes were estimated by the expectation-maximisation algorithm implemented in the Arlequin software.
CD, Crohn's disease; UC, ulcerative colitis.

Acknowledgements

The authors thank Carmen Martínez Cuervo for her expert technical assistance.

C Núñez*
Clinical Immunology Department, Hospital Clínico San Carlos, Madrid, Spain

J Oliver*
Instituto de Parasitología y Biomedicina, CSIC, Granada, Spain

J L Mendoza
Gastroenterology Unit, Hospital Clínico San Carlos, Madrid, Spain

M Gómez-García
Gastroenterology Unit, Hospital Virgen de las Nieves, Granada, Spain

A Piñero
Gastroenterology Unit, Hospital Puerta del Mar, Cádiz, Spain

C Taxonera, M Díaz-Rubio
Gastroenterology Unit, Hospital Clínico San Carlos, Madrid, Spain

M A López-Nevot
Immunology Department, Hospital Virgen de las Nieves, Granada, Spain

E G de la Concha
Clinical Immunology Department, Hospital Clínico San Carlos, Madrid, Spain

A Nieto
Immunology Department, Hospital Puerta del Mar, Cádiz, Spain

E Urcelay, A Martínez*
Clinical Immunology Department, Hospital Clínico San Carlos, Madrid, Spain

J Martín*
Instituto de Parasitología y Biomedicina, CSIC, Granada, Spain

Correspondence to: Dr A Martínez Doncel, Immunology Department, Hospital Clínico San Carlos, C/Martín Lagos, sn 28040 Madrid, Spain; alfmdoncel@terra.es

doi: 10.1136/gut.2007.121905

*These authors contributed equally to this work.

This work was supported by grants SAF2003-08522 and SAF2006-00398. AM has a FIS contract (CP04/00175) and EU works for the ''Fundación para la Investigación Biomédica-Hospital Clínico San Carlos''.

Competing interests: None.

References

1 **Buhner S**, Buning C, Genschel J, *et al*. Genetic basis for increased intestinal permeability in families with Crohn's disease: role of CARD15 3020insC mutation? *Gut* 2006;**55**:342–7.
2 **Monsuur AJ**, de Bakker PI, Alizadeh BZ, *et al*. Myosin IXB variant increases the risk of celiac disease and points toward a primary intestinal barrier defect. *Nat Genet* 2005;**37**:1341–4.
3 **Amundsen SS**, Vatn M, Wijmenga C, *et al*. Association analysis of MYO9B gene polymorphisms and inflammatory bowel disease in a Norwegian cohort. *Tissue Antigens* 2006;**68**:249–52.
4 **van Bodegraven AA**, Curley CR, Hunt KA, *et al*. Genetic variation in myosin IXB is associated with ulcerative colitis. *Gastroenterology* 2006;**131**:1768–74.
5 **Oliver J**, Marquez A, Gomez-Garcia M, *et al*. Association of the macrophage migration inhibitory factor gene polymorphisms with inflammatory bowel disease. *Gut* 2007;**56**:150–1.
6 **van Heel DA**, Fisher SA, Kirby A, *et al*. Inflammatory bowel disease susceptibility loci defined by genome scan meta-analysis of 1952 affected relative pairs. *Hum Mol Genet* 2004;**13**:763–70.
7 **Russell RK**, Satsangi J. IBD: a family affair. *Best Pract Res Clin Gastroenterol* 2004;**18**:525–39.

Cathepsin B gene polymorphism Val26 is not associated with idiopathic chronic pancreatitis in European patients

Pancreatitis is thought to be a disease of autodigestion triggered by premature and intracellular activation of digestive proteases.[1] We and others have shown that lysosomal cathepsin B can activate trypsinogen intracellularly[2] and that a large proportion of pancreatic cathepsin B is physiologically sorted into the secretory compartment.[3] Further support for a role of cathepsin B in pancreatitis came from a recent study published by Mahurkar and coworkers in *Gut*[4] in which the authors reported that a leucine to valine mutation at position 26 of cathepsin B (L26V) is associated with tropical calcifying pancreatitis (odds ratio ~2.2) in patients from southern India. Tropical calcifying pancreatitis is also associated (in up to 50% of cases) with mutations in the SPINK1 gene[5]—with N34S being the most common mutation. In the study by Mahurkar *et al* the cathepsin B L26V mutation was, however, equally as common in SPINK1 N34S patients as in SPINK1 wild type patients, which suggests that cathepsin B is involved in an independent disease causing mechanism for pancreatitis.

As idiopathic chronic pancreatitis in Western countries and tropical calcifying pancreatitis in India share a high prevalence of SPINK1 mutations,[5] we investigated whether the former is also associated with the L26V cathepsin B mutation. We studied 64 patients with idiopathic chronic pancreatitis (ICP, defined as having unequivocal morphological evidence of chronic pancreatitis on computed tomography or endoscopic retrograde cholangio-pancreatography, or both) from northern Germany (aged 3 to 68 years; 38 male, 26 female) and 100 locally recruited healthy control subjects according to a recently reported ethics committee approved protocol.[6] Patients with known risk factors for pancreatitis, such as a history of regular alcohol consumption (more than two drinks or 20 g a day), or with biliary, metabolic, or endocrine disorders, cystic fibrosis, or hereditary pancreatitis were excluded.

Genomic DNA was extracted from blood leucocytes and polymerase chain reaction amplification of exons 2 and 3 of the cathepsin B gene was undertaken using specific oligonucleotides (sense: CGA GAC GGT GCC CCT GTG TGT G; antisense: GAG GCC TTC ACT CTC CCA CTT CC). Sequencing of cathepsin B exons in ICP patients identified 31 heterozygous and 10 homozygous Val26 alleles (allele frequency 0.398), while in the control cohort we found 46 heterozygous and 25 homozygous Val26 mutations (allele frequency 0.48; table 1). To our surprise and in contrast to the study by Mahurkar *et al*,[4] the allele frequency of cathepsin B Val26 appeared to be even higher among controls than among ICP patients.

Being faced with a Val26 allele frequency higher in Western control subjects than in Indian pancreatitis patients, we searched the SNP Genbank of NCBI for ethnic cohorts in whom the frequency of the Val26 variant had been reported. From the reported 16 groups of diverse ethnic backgrounds, the nine largest cohorts (n >40) were selected, comprising 1198 individuals. The according frequencies for C–G mutations (C is replaced by G in Val26) at codon 26 are given in table 2.