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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.

Table 2 Genotype and allele frequencies of *MIF* –173G/C in patients with inflammatory bowel disease and in controls

	Genotypes, (n %)			Alleles, (n %)			
<i>MIF</i> –173G/C	GG	GC	CC	G	C	p Value*	OR (95% CI)
Cohort 1							
Controls (n=361)	275 (76.2)	81 (22.4)	5 (1.4)	631 (87.4)	91 (12.6)		
IBD (n=623)	423 (67.9)	178 (28.6)	22 (3.5)	1024 (82.2)	222 (17.8)	0.002	1.5 (1.1 to 1.9)
CD (n=336)	227 (67.5)	94 (27.9)	15 (4.5)	548 (81.5)	124 (18.4)	0.002	1.5 (1.1 to 2.0)
UC (n=287)	196 (68.3)	84 (29.3)	7 (2.4)	476 (82)	98 (17)	0.02	1.4 (1.04 to 1.9)
Cohort 2							
Controls (n=526)	406 (77.2)	107 (20.3)	13 (2.5)	919 (87.3)	133 (12.6)		
IBD (n=672)	484 (73.2)	165 (23.5)	23 (3.3)	1133 (84.3)	211 (15.7)	0.034	1.2 (1.01 to 1.6)
CD (n=325)	239 (73.5)	78 (24)	8 (2.5)	556 (85.5)	94 (14.4)	0.2	1.1 (0.8 to 1.5)
UC (n=347)	245 (72.9)	87 (23)	15 (3.9)	577 (83.1)	117 (16.8)	0.01	1.4 (1.07 to 1.8)
Combined							
Controls (n=887)	681 (76.7)	188 (21.3)	18 (2)	1550 (87.4)	224 (12.6)		
IBD (n=1295)	907 (70)	343 (26.4)	45 (3.4)	2157 (83.3)	433 (16.7)	0.001	1.3 (1.1 to 1.6)
CD (n=661)	466 (70.5)	172 (26)	23 (3.5)	1104 (83.5)	218 (16.5)	0.002	1.3 (1.1 to 1.6)
UC (n=634)	441 (70.9)	171 (25.7)	22 (3.3)	1053 (83)	215 (17)	0.001	1.4 (1.1 to 1.7)

CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.

*p Value of *MIF* –173* C allele.

Mantel-Haenszel test:

IBD pooled OR 1.37, 95% CI 1.1 to 1.6; p = 0.001.

CD pooled OR 1.34, 95% CI 1.1 to 1.6; p = 0.005.

UC pooled OR 1.4, 95% CI 1.1 to 1.7; p = 0.001.

analysed together, and no significant differences were observed.

Along with the data reported here, the observations that the *MIF* –173* C risk allele is related to high *MIF* expression⁵ and that serum *MIF* levels were markedly increased in patients with IBD^{1–2} support the involvement of *MIF* in the pathogenesis of IBD. Interestingly, transgenic overexpression of *MIF* renders mice markedly more susceptible to experimental colitis.⁶ High levels of *MIF* in patients with IBD might immobilise macrophages and facilitate their localisation at the intestinal mucosa, leading to the production of several inflammatory cytokines. Upregulation of *MIF* in both Crohn's disease and ulcerative colitis indicates that *MIF* might contribute to a broad spectrum of inflammatory responses via innate and acquired immune systems in IBD that may involve different pathogenic mechanisms.⁷

The *MIF* allele frequencies found in the Spanish population are similar to those reported in other white populations.³ It is remarkable that, although an increase in the *MIF* –173* C allele was observed in patients with ulcerative colitis compared with controls, the *MIF* –173* C allele was not associated with susceptibility to ulcerative colitis in the Japanese population.⁸

Regarding the genotype-phenotype analysis, we observed that *MIF* allele frequencies in the different ulcerative colitis and Crohn's disease subgroups were not significantly different when compared with other subphenotypes. It must be cautioned that the numbers in the ulcerative colitis and Crohn's disease subphenotype samples were small and should be expanded before the extent of associations can be properly assessed.

Autoimmune diseases such as IBD are presumed to have common underlying mechanisms and thus, some degree of shared genetic predisposition.⁹ *MIF* genetic variants have been found to be associated with autoimmune diseases.^{3–5} These observations, together with our findings that *MIF* gene polymorphisms are associated with IBD,

provide support for the notion that susceptibility to multiple autoimmune diseases may have some common susceptibility alleles or pathways.

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