PostScript

LETTERS

Iron deficiency anaemia: further education regarding the British Society of Gastroenterology guidelines is required

Kim Gregory, Melanie Halliday, Charles Averill, Nij Bhala, Chris Tselepis, Tariq Iqbal
Department of Gastroenterology, University Hospital of Coventry & Warwickshire, Walsgrave, Coventry, UK

Correspondence to: Dr T Iqbal, Department of Gastroenterology, University Hospital of Coventry & Warwickshire, Walsgrave, Coventry CV2 2DX, UK; tariq.iqbal@uhcw.nhs.uk

doi: 10.1136/gut.2006.117838

The publication of the iron deficiency guidelines in 2005[1] and the adoption of iron deficiency anaemia (IDA) as a 2-week referral criterion by the National Institute for Clinical Excellence lower gastrointestinal (GI) cancer guidelines[2] should have focused attention on this historically poorly managed condition. There is evidence from the primary care literature of poor adherence to previous IDA guidelines.[3] In order to evaulate adherence to the new IDA guidelines in our trust, we undertook a short prospective audit of all patients admitted in 1 month through the medical admissions unit of a large teaching hospital in central England.

The initial phase of this study focused on two questions with regard to diagnosis of anaemia: (1) was serum ferritin measured in patients with microcytic anaemia and (2) were all patients with unexplained IDA assessed serologically for coeliac disease?

We evaluated 995 medical patients admitted during October 2006. Using a haemoglobin cut-off of 13 g/dl for men and 12 g/dl for women, 395 (36%) patients were found to be anaemic, of whom 46 (5%) had microcytosis (<80 fl). Of these, 13 (28%) patients had serum ferritin level measured on the second blood test during hospital stay, and in total 19 (41%) patients with a microcytic anaemia underwent ferritin level estimation during their current admission to hospital. All patients with a low serum ferritin level subsequently proceeded to further GI investigations. Pertinently, none of the patients with microcytic anaemia had blood taken for coeliac disease serology.

We believe that the results of this audit may well be representative of the investigation of anaemia, not just in our trust but also in the secondary care sector generally. The prevalence of adult coeliac disease in western European populations is thought to be about 1 per 100–300 people, based on epidemiological studies in which cohorts of healthy volunteers were screened. Previous case finding studies in primary care[4] have demonstrated that coeliac disease commonly presents with IDA, and, even with the advent of serological testing, is considerably underdiagnosed. Patients presenting for secondary care to different medical and surgical specialties[5] would seem to be at an even higher risk, underlining the importance of education regarding this entity.

Anaemia is an important public health problem, with an estimated prevalence of 2–10%,[6] and although IDA may indicate underlying GI cancer and is associated with seriously detrimental effects on the quality of life,[7] it seems to be poorly managed across the spectrum of healthcare. We believe it is important to raise awareness of the British Society of Gastroenterology IDA guidelines to promote better diagnosis, investigation and management of this common public health problem.

Competing interests: None declared.

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*Association of Bcl*I polymorphism of the glucocorticoid receptor gene locus with response to glucocorticoids in inflammatory bowel disease

Glucocorticoids (GCs) are immunosuppressive drugs used for the acute treatment of patients with moderate to severe inflammatory bowel disease (IBD),[1] but interindividual variability in the response to these agents is frequently observed.[2] GCs diffuse freely into cells and bind to an intracellular receptor (hGR/NR3C1), so the sensitivity to these drugs may depend on the receptor number and affinity or on their availability to the receptors, and transport proteins (including P-glycoprotein (Pgp) encoded by the MDR1/ABCB1 gene) can modify their intracellular concentration.[3 4] Polymorphisms in the hGR and MDR1 genes have been described in different populations and may contribute to the variability in sensitivity to GCs observed in the clinical setting.[3 4]

A study was conducted to estimate the impact of genetic variations in hGR and MDR1 genes on the efficacy and individual response to GCs in young patients with IBD. Polymorphisms of the hGR gene (*Bcl*I and N363S which are related to GC hypersensitivity and ER22/23EK which is associated with relative resistance to GCs[5]) and the MDR1 gene (C3435T and G2677T which are associated with changes in Pgp expression and activity[6]) were studied in 119 young patients (58 female) with IBD (64 with Crohn's disease and 55 with ulcerative colitis) of mean (SD) age 11.7 (5.94) years at the onset of the disease and 100 consecutive healthy blood donors from the same geographical area using PCR-RFLP.[6–8] Patients were enrolled between July 2000 and March 2005 in the gastroenterology units of the Children's Hospitals of Trieste, Firenze and Genoa, Italy. The study included all consecutive patients with IBD who had been treated with GCs for at least 30 days (prednisone 1–2 mg/kg/day for 2–4 weeks and subsequent dose tapering by 5 mg/week to a dose of 20 mg and then by 2.5 mg/week below 20 mg) and had a minimum follow-up period of 1 year. They were divided into two groups based on their response to GC treatment: GC-dependence (n = 45 patients) was defined by an initial response to prednisone with relapse on dose reduction, not allowing steroid discontinuation,[9] and GC-responsiveness (n = 67 patients), equivalent to therapeutic success, was defined as GC withdrawal without the need for steroids for at least 1 year.[10] Six patients (5.0%) initially had a partial response to GCs but steroid treatment was continued with the addition of azathioprine and aminosalicylates, so these patients were included in the study. Seven patients (5.9%) exhibited complete resistance to GCs and required colectomy; genetic analysis was also performed on this group and is reported in table 1. These patients were not considered in the statistical analysis; however, their number was very low, similar to that described by other authors in children,[11] and, in addition, they did not complete the scheduled observation period.

The genotype frequencies for all tested polymorphisms were consistent with the Hardy-Weinberg equilibrium in both the study and control groups. A significantly higher frequency of *Bcl*I mutated genotype, associated with GC hypersensitivity, was observed in the GC-responsive patients than in the GC-dependent group (OR 0.15, 95% CI 0.03 to 0.68, p = 0.0075/multiple testing corrected p = 0.0375) or controls (OR 3.61, 95% CI 1.44 to 9.01, p = 0.006/0.030). The significance was lost when the association was studied separately in patients with Crohn's disease (OR 0.23, 95% CI 0.045 to 1.13, p = 0.10/0.5) and ulcerative colitis (OR 0.10, 95% CI 0.005 to 1.97, p = 0.065/0.33) because of the small patient sample. No differences were observed for the other polymorphisms considered (table 1). A significantly higher frequency of the *Bcl*I polymorphism was also evident in patients with Crohn's disease than in healthy volunteers (OR 2.93, 95% CI 1.14 to 7.54, p = 0.03/0.06), confirming a previous observation in a smaller number of subjects (table 1).[12]

A logistic regression model, in which the response to GCs was the dependent variable and patients' age, sex, type of IBD and the considered genotypes were the independent variables, confirmed an independent significant association between the response to GCs and the variable *Bcl*I genotype (table 2).

Table 1 Frequency of MDR1 and hGR polymorphisms in GC-dependent, GC-responsive and GC-resistant patients with inflammatory bowel disease and frequency of *BclI* polymorphism in patients with Crohn's disease and ulcerative colitis and controls

	Genotype			OR (95% CI)	p Value
N363S	WT (%)	HET (%)	MUT (%)	HET vs WT	
GC-dependent (n = 45)	41 (91.1)	4 (8.9)	0 (0.0)	1.21 (0.31 to 4.78)	1.00*/1.00†
GC-responsive (n = 67)	62 (92.5)	5 (7.5)	0 (0.0)		
GC-resistant (n = 7)	7 (100.0)	0 (0.0)	0 (0.0)		
BclI	WT (%)	HET (%)	MUT (%)	MUT vs WT + HET	
GC-dependent (n = 45)	19 (42.2)	24 (53.3)	2 (4.5)	0.15 (0.03 to 0.68)	0.0075*/0.0375†
GC-responsive (n = 67)	27 (40.3)	24 (35.8)	16 (23.9)		
GC-resistant (n = 7)	4 (57.1)	3 (42.9)	0 (0.0)		
ER22/23EK	WT (%)	HET (%)	MUT (%)	HET vs WT	
GC-dependent (n = 45)	43 (95.6)	2 (4.4)	0 (0.0)	0.57 (0.10 to 3.06)	0.70*/1.00†
GC-responsive (n = 67)	62 (92.5)	5 (7.5)	0 (0.0)		
GC-resistant (n = 7)	7 (100.0)	0 (0.0)	0 (0.0)		
C3435T	WT (%)	HET (%)	MUT (%)	MUT vs WT + HET	
GC-dependent (n = 45)	10 (22.2)	23 (51.1)	12 (26.7)	1.07 (0.45 to 2.53)	1.00*/1.00†
GC-responsive (n = 67)	16 (23.9)	34 (50.7)	17 (25.4)		
GC-resistant (n = 7)	4 (57.1)	1 (14.3)	2 (28.6)		
G2677T	WT (%)	HET (%)	MUT (%)	MUT vs WT + HET	
GC-dependent (n = 45)	12 (26.7)	27 (60.0)	6 (13.3)	0.53 (0.19 to 1.50)	0.32*/1.00†
GC-responsive (n = 67)	21 (31.3)	31 (46.3)	15 (22.4)		
GC-resistant (n = 7)	4 (57.1)	2 (28.6)	1 (14.3)		
BclI	WT (%)	HET (%)	MUT (%)	MUT vs WT + HET	
Controls (n = 100)	39 (39.0)	53 (53.0)	8 (8.0)	–	–
CD (n = 64)	23 (35.9)	28 (43.7)	13 (20.3)	2.93 (1.14 to 7.54)	0.03*/0.060†
UC (n = 55)	27 (49.1)	22 (41.8)	5 (9.1)	1.15 (0.36 to 3.70)	0.77*/1.00†

GC, glucocorticoid; WT, wild type; HET, heterozygous; MUT, mutated; CD, Crohn's disease; UC, ulcerative colitis.
Odds ratios (OR) and p values calculated for GC-dependent vs GC-responsive.
*p values calculated from Fisher's exact test.
†p values calculated after adjustment for multiple comparison by Bonferroni correction.

Table 2 Odds ratios with confidence intervals and p values for the independent variables in the logistic regression model for the response to steroid treatment (GC-dependent vs GC-responsive)

Variable	OR (95% CI)	p Value
Age (years)	1.07 (0.98 to 1.17)	0.10
Sex		
Female	1.00	
Male	1.89 (0.83 to 4.35)	0.14
Type of IBD		
CD	1.00	
UC	1.04 (0.44 to 2.44)	0.93
N363S		
WT	1.00	
HET	1.32 (0.30 to 5.82)	0.72
BclI		
WT+HET	1.00	
MUT	5.94 (1.23 to 28.6)	0.026*
ER22/23EK		
WT	1.00	
HET	2.38 (0.42 to 13.5)	0.32
C3435T		
WT+HET	1.00	
MUT	0.56 (0.15 to 2.08)	0.38
G2677T		
WT+HET	1.00	
MUT	2.56 (0.54 to 11.0)	0.23

IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; WT, wild type; HET, heterozygous; MUT, mutated.

This study shows that patients with mutated *Bcl*I genotype, which is associated with GC hypersensitivity, respond better to GC treatment and are less likely to need additional courses of steroid treatment. The mechanism of this effect is unclear at present. This polymorphism is intronic and not located in a coding, regulatory or splicing region of the hGR gene, but it may be linked to variations in the promoter region or 3′ untranslated region of the gene, or could act as a marker of other functionally important polymorphisms in the vicinity.[13] Although this is the first study of the effects of hGR polymorphisms on sensitivity to GCs in IBD, previous work with the polymorphic hGR and MDR1 genotypes in other diseases has produced conflicting results, suggesting that the influence of these polymorphisms may vary depending on tissue type, disease and ethnicity.[14]

Given the high incidence of a suboptimal response to GCs in the treatment of IBD and the frequency of side effects, the identification of subjects who are likely to respond poorly to these agents would be very useful, and these patients should be treated earlier with steroid-sparing drugs. The *Bcl*I polymorphism could therefore be a useful molecular marker to identify patients responsive to GC treatment.

Sara De Iudicibus, Gabriele Stocco
Department of Biomedical Sciences, University of Trieste, and Department of Reproductive and Developmental Sciences and IRCCS, Istituto per l'Infanzia Burlo Garofolo, Clinica Pediatrica, Trieste, Italy

Stefano Martelossi
Department of Reproductive and Developmental Sciences and IRCCS, Istituto per l'Infanzia Burlo Garofolo, Clinica Pediatrica, Trieste, Italy

Ilenia Drigo
Department of Biomedical Sciences, University of Trieste, and Department of Reproductive and Developmental Sciences and IRCCS, Istituto per L'Infanzia Burlo Garofolo, Clinica Pediatrica, Trieste, Italy

Stefania Norbedo
Department of Reproductive and Developmental Sciences and IRCCS, Istituto per l'Infanzia Burlo Garofolo, Clinica Pediatrica, Trieste, Italy

Paolo Lionetti, Elena Pozzi
Research Children's Hospital 'Meyer', Florence, Italy

Arrigo Barabino
Research Children's Hospital 'Gaslini', Genoa, Italy

Giuliana Decorti
Department of Biomedical Sciences, University of Trieste, Italy

Fiora Bartoli, Alessandro Ventura
Department of Reproductive and Developmental Sciences and IRCCS, Istituto per l'Infanzia Burlo Garofolo, Clinica Pediatrica, Trieste, Italy

Correspondence to: Dr Giuliana Decorti, Department of Biomedical Sciences, University of Trieste, Via L Giorgieri 7, 9, 34127 Trieste, Italy; decorti@units.it

doi: 10.1136/gut.2006.116160

This research was supported by grants from the Italian Ministry of University and Scientific Research (PRIN project 2005065797). SDI, GS and ID are recipients of a research fellowship from IRCCS Burlo Garofolo, Trieste.

Competing interests: None.

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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.