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Allelic Variations of the Multidrug Resistance Gene Determine Susceptibility and Disease Behavior in Ulcerative Colitis

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Background & Aims: **The *MDR1* gene encodes P-glycoprotein 170, an efflux transporter that is highly expressed in intestinal epithelial cells. The *MDR1* exonic single nucleotide polymorphisms (SNPs) C3435T and G2677T have been shown to correlate with activity/expression of P-glycoprotein 170.** ***Methods:*** **This was a case-control analysis of *MDR1* C3435T and G2677T SNPs in a large well-characterized Scottish white cohort (335 with ulcerative colitis [UC], 268 with Crohn's disease [CD], and 370 healthy controls). We conducted 2-locus haplotype and detailed univariate and multivariate genotypic-phenotypic analyses.** ***Results:*** **The *MDR1* 3435 TT genotype (34.6% vs 26.5%; $P = .04$; odds ratio [OR], 1.60; 95% confidence interval [95% CI], 1.04–2.44) and T-allelic frequencies (58.2% vs 52.8%; $P = .02$; OR, 1.28; 95% CI, 1.03–1.58) were significantly higher in patients with UC compared with controls. No association was seen with CD. The association was strongest with extensive UC (TT genotype: 42.4% vs 26.5%; $P = .003$; OR, 2.64; 95% CI, 1.34–4.99; and T allele: 63.9% vs 52.8%; $P = .009$; OR, 1.70; 95% CI, 1.24–2.29), and this was also confirmed on multivariate analysis ($P = .007$). The G2677T SNP was not associated with UC or CD. These 2 SNPs lie in linkage disequilibrium in our population (D', .8–.9; r^2, .7–.8). Two-locus haplotypes showed both positive (3435T/G2677 haplotype: $P = .03$; OR, 1.44) and negative (C3435/2677T haplotype: $P = .002$; OR, .35) associations with UC. Homozygotes for the haplotype 3435T/G2677 were significantly increased in UC ($P = .017$; OR, 8.88; 95% CI, 1.10–71.45).** ***Conclusions:*** **Allelic variations of the *MDR1* gene determine disease extent as well as susceptibility to UC in the Scottish population. The present data strongly implicate the C3435T SNP, although the 2-locus haplotype data underline the need for further detailed haplotypic studies.**

Ulcerative colitis (UC) and Crohn's disease (CD) are common, chronic inflammatory disorders of the intestines characterized by a dysregulated mucosal immune response.[1] Epidemiologic and linkage studies suggest that genetic factors play a significant role in determining susceptibility to inflammatory bowel disease (IBD).[2] Genetic linkage analyses, through genome-wide screens, have identified a number of susceptibility loci, revealing the complexity of IBD.

Recent attention has focused on the multidrug resistance 1 (*MDR1*) gene and its product, P-glycoprotein 170, as a potential determinant of susceptibility to IBD.[3] P-glycoprotein 170, which functions as an adenosine triphosphate–dependent efflux transporter pump, is highly expressed in the epithelial surfaces of the intestine, biliary ductules, proximal tubules of kidneys, and central nervous system, where it forms the basis of the blood-brain barrier.[4–6] Interindividual variability of P-glycoprotein expression in the intestine plays a role in determining the pharmacokinetics of a wide-ranging number of substrates. Nevertheless, the exact physiologic role in the gut remains unknown. The high constitutive levels of expression of P-glycoprotein 170 in the gut suggest a role in protection not only against xenobiotics but also bacterial products.

The *MDR1* gene is an attractive candidate gene for IBD for several reasons.[3] First, mdr-1a–deficient mice develop a UC-like phenotype when maintained in a specific pathogen–free environment that is reversed with antibiotics.[7] Bone marrow transfer studies show that these mice develop colitis primarily due to deficiency of mdr-1 in the epithelial rather than the lymphoid cells. Second, the *MDR1* gene maps to chromosome 7q22, which has been identified as a putative locus of susceptibility for IBD by genome-wide scanning in a UK cohort.[8] Recent subsequent meta-analysis of all genome-wide scans confirms suggestive linkage to this region.[9] Most recently, compelling data by Langmann et al have shown that *MDR1* gene expression is down-regulated in IBD with expression significantly reduced in the colonic tissue of patients with UC but not CD.[10] In contrast to

Abbreviations used in this paper: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

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this, Farrell et al have suggested that increased P-glycoprotein 170 expression may be associated with failure of medical therapy in IBD.[11]

The *MDR1* gene is composed of 28 exons and is 209 kilobases in length, and 29 single nucleotide polymorphisms (SNPs) have been described.[12–14] Two SNPs, exonic variant C3435T and G2677T/A, have been shown to correlate with activity/expression of P-glycoprotein 170. The C3435T SNP in exon 26 has been most extensively investigated and was first shown to correlate with expression of P-glycoprotein 170.[12] In this study, the TT genotype was associated with decreased intestinal P-glycoprotein 170 expression with functional consequence as inferred by increased digoxin uptake following oral administration.[15] This has been replicated in other pharmacokinetic studies.[16–18] In addition, the effect of the *MDR1* C3435T SNP and its postulated correlation with P-glycoprotein 170 activity/expression has been shown to play a role in drug-resistant epilepsy,[19] immune recovery after initiation of antiretroviral therapy in human immunodeficiency virus,[20] and the development of renal cell carcinoma.[21]

The G2677T/A SNP in exon 21 results in 2 distinct amino acid changes, namely 893Ser (G2677T) or the much rarer 893Thr (G2677A), and has been shown to be associated with altered transporter function or expression.[17,22] Several studies have suggested that the C3435T and G2677T SNP may lie in linkage disequilibrium.[17,23–25]

Recently, Schwab et al in Germany suggested that both T allele and TT genotype were associated with increased susceptibility in UC but not CD.[26] Subsequent replication studies involving this SNP alone in other centers have been inconsistent.[27,28] In a North American study examining both G2677T and C3435T SNPs (and, additionally, a C1236T variant in exon 12), Brant et al[29] showed an association only between the Ala893 polymorphism (G2677) and IBD, although these investigators did not specify whether the association was with CD or UC. A significant association was observed from both a case-control study and a pedigree disequilibrium test with IBD. The investigators proposed that the G allele (Ala893) might confer a genetic risk factor to susceptibility of IBD.

We aimed firstly to investigate the contribution of the *MDR1* C3435T and G2677T SNP in a large, independent, well-characterized population of Scottish white people. We determined whether 2-locus haplotypes provide a stronger association with disease than individual SNPs to resolve the controversies regarding the contribution of this gene to disease susceptibility. We rigorously conducted a detailed subphenotypic review of all subjects genotyped to assess whether these variants are particularly implicated in determining disease extent and behavior in IBD. To clarify whether *MDR1* genotypes may influence drug responsiveness in UC, we further subcategorized our patients to the phenotypes of severe disease and need for surgery in UC.

Table 1. Demographics and Clinical Characteristics of Patients With UC (n = 335)

Sex (M/F)	184/151
Age at onset (*y*)	35.0 (25.3–50.3)
Age at onset younger than 16 years (%)	10 (3)
Smoking history (%)	
Current	28 (8.5)
Ex-smoker	135 (40.4)
Never	172 (51.4)
Ethnicity	
Scottish white (%)	99
Other	1 Jewish, 1 Japanese, and 2 Asian persons
Disease extent	
Extensive disease (beyond the splenic flexure)	118 (35.2)
Left-sided colitis	149 (44.5)
Proctitis	68 (20.3)
Severe disease[a]	113 (33.7)
Surgery for severe disease	63 (18.8)
Extraintestinal manifestations (%)	31 (9.4)
Primary sclerosing cholangitis (%)	6 (1.8)
Azathioprine therapy (%)	48 (14.2)

[a]Severe UC defined as patients who developed a severe acute attack of UC satisfying the Truelove and Witts criteria.

Patients and Methods

Patients

This study was approved by the Lothian Research and Ethics Committee, and written consent was obtained from all patients. A total of 335 patients with UC and 268 with CD were recruited from the Lothian region (Scotland). The diagnosis of IBD was determined by standard clinical, radiologic, endoscopic, and histologic criteria.

Tables 1 and 2 summarize the clinical characteristics of patients studied. The median ages at diagnosis of UC and CD were 35.0 years (interquartile range, 25.3–50.3 years) and 26.6 years (interquartile range, 19.9–37.0 years), respectively. The ethnicity of our study population was predominantly Scottish white (99%). There were more men in the UC cohort (54.5%) than in the CD cohort (43.5%).

Phenotypic Assessment

UC phenotype was classified by disease extent, disease severity, and need for surgery. The extent of disease was documented at the time of latest follow-up. We defined extensive disease as disease extending beyond the splenic flexure, left-sided colitis as disease extending to the splenic flexure, and proctitis as disease limited to the rectum as determined by histologic and macroscopic evidence. In discordant cases, the histologic evidence was used. Patients who had developed an

Table 2. Demographics and Clinical Characteristics of Patients With CD

Sex (M/F)	117/151
Smoking history (%)	
Current	68 (25.4)
Ex-smoker	77 (28.7)
Never	123 (45.9)
Age at onset (*y*)	26.6
Median (interquartile range)	(19.9–37.0)
Age at onset	203
No. (%) (younger than 40 years, A1)	(75.7)
Ethnicity	
Scottish white (%)	99
Other	(1 Jewish and 2 Asian persons)
Drug therapy (%)	
Infliximab	45 (16.8)
Azathioprine	106 (39.5)
Disease location (%) (n = 240)	
Ileal (L1)	83 (30.9)
Colonic (L2)	118 (39.9)
Ileocolonic (L3)	57 (21.3)
Upper gastrointestinal (L4)	21 (7.9)
Disease behavior *at diagnosis* (%)	
Inflammatory (B1)	196 (73.1)
Stricturing (B2)	21 (7.8)
Penetrating (B3)	51 (19.0)
Disease behavior at latest follow-up (%)	
Inflammatory (B1)	95 (35.4)
Stricturing (B2)	43 (16.1)
Penetrating (B3)	130 (48.4)
Surgery (%)	122 (45.8)
Extraintestinal manifestations (%)	59 (22.1)

NOTE. Disease location, behavior, and age at onset were defined according to the Vienna classification.

acute severe attack of UC (satisfying the Truelove and Witts criteria) requiring intensive inpatient medical therapy were regarded to have severe UC. Within the category of severe UC, a further subset of patients who had failed to respond to medical therapy and consequently required surgery were categorized under the phenotype "need for surgery." Other phenotypic details such as smoking, family history, presence of primary sclerosing cholangitis, and other extraintestinal manifestations were also recorded.

CD was classified according to the Vienna classification of disease location, behavior, and age at diagnosis as previously described.[30] We have previously shown that disease behavior is not stable over time and therefore have analyzed disease behavior only at time of diagnosis and at latest follow-up.[31]

Controls

A total of 370 healthy controls, comprised of actively recruited healthy subjects (n = 105) and blood donors (n = 265), were all recruited from the Lothian region between 2000 and 2002. There were no differences in the demographics between these 2 groups. There were 179 men and 191 women, and the median age at recruitment was 37.1 years (interquartile range, 25.9–47.0 years).

Genotyping

Genotyping was performed using TaqMan (ABI, San Diego, CA). TaqMan probes were available from ABI-assay-on-demand/design: C3435T (rs1045642) and G2677T/A (rs2032582). Sequence and reaction settings are available on request.

G2677T/A is a triallelic SNP, with reported frequencies of the rare A allele in European white people ranging from undetected to 4%.[13,17,32–34] We sequenced 200 chromosomes of the UC group (100 individuals) and confirmed a G2677A allelic frequency of only 2% in our population. In view of this low frequency, we chose to genotype the 2 common variants of the G2677T/A SNP using TaqMan reaction.

Data Analysis

Genotype and allelic frequencies between cases and controls were compared using a 2 × 2 table and Fisher exact test. Odds ratios (ORs) are given with 95% confidence intervals (CIs) and 2-sided P values. $P \leq .05$ was considered significant. All calculations were performed using the Graph Pad Instat program (Graph Pad Software, San Diego, CA). Fisher exact test was used to evaluate if the homozygote and heterozygote frequencies for each SNP deviate from the Hardy–Weinberg equilibrium. Two-locus haplotype frequencies were measured using the expectation-maximization algorithm utilizing the SNPHAP program (bioinformatic programs available and accessed via the Medical Research Council–Rosalind Franklind Centre of Genomic Research Web site: http://www.rfcgr.mrc.ac.uk).[35]

Multivariate analysis was performed using a logistic regression model to test the association between phenotype and genotypes. Two methods were used for haplotypic association with disease: (1) the log-likelihood ratio method, in which inferred haplotypes were compared in cases, controls, and cases/controls combined, and (2) directly comparing the haplotype frequencies between cases and controls. The log-likelihood ratio tests whether a model in which haplotype frequencies in cases are different from controls or a model in which there are no differences between cases and controls fit the data obtained better.[35] Significance for association was calculated using the test statistic $2*ln(Lcase) + ln(Lcontrol) - ln(Lcase/Lcontrol)$, which has a χ^2 distribution with n − 1 *df,* where n is the number of inferred haplotypes. We measured the linkage disequilibrium between SNPs using Cocaphase software (bioinformatic programs available and accessed via the Medical Research Council–Rosalind Franklind Centre of Genomic Research Web site: http://www.rfcgr.mrc.ac.uk).

The log-likelihood analysis and direct haplotype comparison are complementary methods to detect association in haplotypic data sets. Log-likelihood analysis is used specifically to address the problem of phase uncertainty encountered when direct comparisons are made from haplotype frequencies that are inferred. Direct haplotype comparison assumes that all haplotypes are known without error and therefore can be counted. However, there remains a degree of uncertainty even in the

Table 3. Genotype and Allelic Frequencies of *MDR1* C3435T Polymorphism in UC and CD Compared With Controls

	CC (%)	CT (%)	TT (%)	C (%)	T (%)	TT vs CC/OR/95% CI	T vs C/OR/95% CI
UC (n = 335)	61 (18.2)	158 (47.2)	116 (34.6)	280 (41.8)	390 (58.2)	.04/1.60/1.04–2.44	.02/1.28/1.03–1.58
CD (n = 268)	56 (20.9)	140 (52.2)	72 (26.9)	252 (47.0)	284 (53.0)	.81/1.08/.68–1.69	.43/1.03/.83–1.29
Healthy controls (n = 370)	82 (22.2)	190 (51.3)	98 (26.5)	354 (47.8)	386 (52.8)		

NOTE. UC vs CD: TT genotype, $P = .12$; OR, 1.48; 95% CI, .93– 2.36; T allele, $P = .07$; OR, 1.24; 95% CI, .98–1.24.

case of estimated diplotypes. Although the likelihood-based method will test for association, it does not provide an estimate of the size of the effect; for this, we used the diplotypes most likely to do so.

Results

Effect of *MDR1* C3435T and G2677T Polymorphism on Overall Disease Susceptibility

Both the T allele and TT genotype of the *MDR1* 3435 SNP were significantly increased in patients with UC (58.2% vs 52.8%; $P = .02$; OR, 1.28; 95% CI, 1.03–1.58) compared with healthy controls (34.6% vs 26.5%; $P = .04$; OR, 1.60; 95% CI, 1.04–2.44) (Table 3). No significant differences in allele or genotype frequencies were seen in patients with CD (53.0% vs 52.8% [$P = .43$] and 26.9% vs 26.5% [$P = .81$], respectively) when compared with controls. A trend toward higher T-allele and genotype frequencies in UC was observed when compared with CD (58.2% vs 53.0% [$P = .12$] and 34.6% vs 26.9% [$P = .07$]).

We did not detect any significant differences in carriage rate (presence of one or 2 copies of alleles) of 3435T in the 3 groups (UC, 81.8%; CD, 79.1%; healthy controls, 77.8%). Our data suggest that the TT genotype rather than T-allele carriage plays the more significant role in the association with UC. The OR, compared with the CC genotype, for the TT genotype was 1.59 (95% CI, 1.04–2.44) and for the CT genotype was 1.12 (95% CI, .76–1.66). Therefore, a stronger significance was also obtained when we compared the homozygosity rate (TT genotype/non-TT genotype) in UC ($P = .02$; OR, 1.47; 95% CI, 1.06–2.03).

No significant differences were observed for allelic and genotype frequencies for *MDR1* G2677T polymorphism on overall disease susceptibility for either UC or CD (Table 4). There was no overall association with IBD in our population for the G2677T SNP ($P = .26$), although the G2677 allele frequency showed a trend to be higher in patients with UC compared with controls (54.6% and 51.2%, respectively). All genotype frequencies in both cases and controls were consistent with Hardy–Weinberg equilibrium.

In addition to this, we performed a sex-matched analysis for both C3435T and G2677T SNP with controls (335 and 268 healthy controls with 48.5% and 43.5% men for UC and CD, respectively). The association observed with C3435T SNP and UC remained significant, with the TT genotype ($P = .05$; OR, 1.58; 95% CI, 1.02–2.45) and T allele ($P = .03$; OR, 1.26; 95% CI, 1.03–1.06) both significantly increased. No other significant differences were detected in sex-matched analysis (full details available on request).

Linkage Disequilibrium Between C3435T and G2677T Polymorphisms

The C3435T and G2677T SNP are in linkage disequilibrium with each other in our population (cases, $D' = .8$ and $r^2 = .7$; controls, $D' = .9$ and $r^2 = .8$). Therefore, we proceeded to perform 2-locus haplotype association tests with UC and CD.

The Effect of 2-Locus Haplotype (C3435T/G2677T) on Disease Susceptibility

Using the log-likelihood ratio test as described earlier, we were able to show an association of 2-locus haplotypes of C3435T/G2677T with UC ($P = .0056$, 3 *df*). On single haplotype analysis, the carriage of 3435T/2677G haplotype conferred an increased risk for UC (OR, 1.44; $P = .03$; 95% CI, 1.03–1.99) (Table 5). In

Table 4. Genotype and Allele Frequencies of *MDR1* G2677T Polymorphism in UC and CD Compared With Controls

	GG (%)	GT (%)	TT (%)	G (%)	T (%)	GG vs TT/OR/95% CI	G vs T/OR/95% CI
UC (n = 335)	95 (28.3)	176 (52.5)	64 (19.1)	366 (54.6)	304 (45.4)	.16/1.37/.89–2.09	.19/1.15/.93–1.42
CD (n = 268)	75 (27.9)	133 (47.8)	60 (22.4)	283 (52.8)	253 (47.2)	.57/1.15/.74–1.79	.57/1.07/.86–1.34
Healthy controls (n = 370)	102 (27.6)	174 (47.0)	94 (25.4)	378 (51.2)	362 (49.8)		

NOTE. UC vs CD: GG genotype, $P = .48$; OR, 1.19; 95% CI, .75– 1.89; G allele, $P = .19$; OR, 1.15; 95% CI, .93–1.89.

Table 5. Two-Locus Haplotypes of C3435T/G2677T SNPs With UC and CD

Haplotype	UC (%)	Healthy Controls (%)	*P* value/OR/95% CI	CD (%)	Healthy Controls (%)	*P* value/OR/95% CI
3435T/2677T	291/379 (43.4)	332/408 (44.9)	.59/.94/.76–1.16	219/317 (40.8)	332/408 (44.9)	.16/.85/.67–1.06
C3435/G2677	276/394 (41.2)	300/440 (40.6)	.82/1.03/.83–1.27	221/315 (41.2)	300/440 (40.6)	.82/1.03/.82–1.29
3435T/G2677	91/579 (13.5)	73/667 (9.8)	.03/1.44/1.03–1.99	65/471 (12.1)	73/667 (9.8)	.20/1.26/.88–1.79
C3435/2677T	11/659 (1.7)	34/706 (4.6)	.002/.35/.17–.69	35/501 (5.9)	34/706 (4.6)	.13/1.45/.89–2.36

NOTE. Contingency tests were used to test the association of the inferred haplotypes in the groups of UC and CD, respectively, as compared with the control group (total number of a particular haplotype/total number of remaining haplotypes compared between cases and controls with *P* values and 95% CIs given).

contrast, carriage of the 3435C/2677T haplotype was associated with a protective effect in UC (OR, .35; $P = .002$; 95% CI, .17–.69). Similar trends were observed with the same haplotypes in CD, but these failed to reach significance (3435C/2677T allele, $P = .20$; 3435T/2677G allele, $P = .13$).

It is noteworthy that a significantly higher number of patients with UC were homozygotes for both TT 3435 and GG 2677 compared with a low frequency in healthy controls (8 patients possessing 3435TT and GG 2677 genotype vs 1; $P = .017$; OR, 8.88; 95% CI, 1.10–71.45). A similar trend was observed in patients with CD (5 patients possessing 3435TT and GG 2677 genotype; $P = .08$; OR, 6.82; 95% CI, .79–58.77). Combining UC and CD yielded a greater significance ($P = .013$; OR, 8.34; 95% CI, 1.09–64.07). There were no obvious associations observed in simple and compound heterozygotes of either UC or CD with healthy controls.

Because carriers of double homozygote mutants were uncommon (14 individuals in total), we analyzed the phenotypes of these patients. In UC, 6 of 8 patients had pancolitis, of whom 4 required surgery as a consequence of severe disease. Of the 5 patients with CD carrying these 2 mutations, one patient had colonic disease (L2), 3 had ileocolonic disease (L3), and one had ileal disease (L1); 3 of 5 of these patients required surgery for active disease.

Genotype-Phenotype Analysis: Univariate Analysis

UC and CD. The T allele and TT genotype of the *MDR1* C3435T showed a highly significant association with the phenotype of extensive disease (OR, 1.70; 95% CI, 1.24–2.29; $P = .009$ and OR, 2.64; 95% CI, 1.34–4.99; $P = .0027$, respectively). Both the frequencies of T allele and TT genotype of *MDR1* C3435T were increased in patients with left-sided colitis and proctitis, but these differences were not significant. A significantly higher T-allele frequency was also observed in patients with severe disease (OR, 1.39; $P = .04$; 95% CI, 1.02–1.88). Although the T-allele and TT-genotype frequencies were even higher in the subgroup of patients who had required surgery for failure of medical treatment for severe UC (T-allele frequency, 60.3%; TT genotype, 41.3%), significance was not achieved in this smaller group (Table 6).

Interestingly, for the *MDR1* G2677T SNP, trends of association to severe disease and surgery were observed for the GG genotype and G allele. In patients with severe disease who required surgery, the GG-genotype frequency was 41.2% compared with 27.5% of controls ($P = .11$; OR, 1.85; 95% CI, .89–3.85). This trend was not observed in the subgroup of patients with extensive disease.

We did not observe any associations with subphenotypic categories of CD (data not shown); specifically, colonic CD was not associated with either SNP (24.7% TT-genotype frequency vs 26.5% for controls; $P = .9$).

Multivariate Analysis

The phenotypes of disease extent, disease severity, and need for surgery were considered in our multivariate model. Multivariate analyses show that the TT genotype of *MDR1* 3435 remained significantly associated with extensive disease ($P = .007$). The phenotypes of severe disease and surgery were not significant ($P = .8$ and $P =$

Table 6. Genotype-Phenotype Analysis for C3435T and G2677T SNP in UC

	C3435T					G2677T				
Genotypes	CC (%)	CT (%)	TT (%)	TT genotype: *P* value/OR/95% CI	T-allele frequency: *P* value/OR/95% CI	GG (%)	GT (%)	TT (%)	GG genotype: *P* value/OR/95% CI	G-allele frequency: *P* value/OR/95% CI
Extensive UC	17 (14.4)	51 (43.2)	50 (42.4)	.003/2.64/1.34–4.99	.009/1.70/1.24–2.29	32 (27.1)	60 (50.9)	26 (22.0)	.76/1.13/.63–2.04	.71/1.06/.79–1.42
Left-sided disease	30 (20.1)	74 (49.7)	45 (30.2)	.49/1.25/.73–2.17	.41/1.12/.86–1.47	44 (29.5)	80 (53.7)	25 (16.8)	.12/1.62/.92–2.85	.13/1.24/.94–1.62
Proctitis	14 (20.6)	37 (54.4)	21 (30.9)	.58/1.25/.60–2.62	.57/1.11/.79–1.59	19 (27.9)	36 (52.9)	13 (19.1)	.57/1.35/.63–2.88	.51/1.14/.79–1.65
Severe disease	23 (20.3)	44 (38.9)	46 (40.7)	.09/1.67/.94–2.99	.04/1.39/1.02–1.88	35 (31.0)	53 (47.8)	20 (21.2)	.17/1.61/.87–2.99	.14/1.27/.93–1.72
Surgery for severe disease	13 (20.6)	24 (38.1)	26 (41.3)	.21/1.67/.81–3.46	.18/1.31/.89–1.91	26 (41.2)	24 (38.1)	13 (20.6)	.11/1.84/.89–3.79	.06/1.46/.99–2.14

.4, respectively). Other models, which included age at onset, smoking status, presence of primary sclerosing cholangitis, and extraintestinal manifestations, showed no additional associations. No associations were observed with CD.

Discussion

This study firstly provides replicated confirmation for the association of the *MDR1* C3435T SNP with UC. In addition, we have made novel observations with respect to genotype-phenotype correlations, notably the strong association of the C3435T SNP with extensive UC. Finally, the haplotypic analyses involving C3435T and G2677T SNPs provide further new insights into the complexities of the contribution of the *MDR1* gene; both protective and susceptible haplotypes were identified.

Indeed, in view of the strength of the phenotypic associations identified in the present study, it is of interest to reconsider whether the positive association with C3435T SNP seen in the study by Schwab et al[26] (43% of patients had extensive UC in that cohort) may have been driven by this phenotype. A significant association with extensive UC was not detected in that study, but that may have been due to lack of statistical power in a smaller subgroup (n = 63).

Obvious care has been taken in other studies that have examined these candidate SNPs (C3435T and G2677T/A) by using both case-control and family-based association designs.[27,29] Croucher et al reported no association of the C3435T SNP with UC or CD in German and British populations.[27] In the North American study, in which the C3435T, G2677T/A, and C1236T (exon 12) SNPs were investigated, only the G2677 allele was associated with IBD.[29] In that study, Brant et al showed that the G2677 allele (Ala893) was significantly higher in patients with IBD compared with controls (G-allele frequency: 61.5% in UC vs 56.5%; $P = .002$). Significant association was also seen in pedigree disequilibrium transmission only for the subset with CD.

Several factors are pertinent when considering the apparently inconsistent results from these studies. The descriptions of the phenotypic details of the cohorts were lacking, and our data have now emphasized the importance of phenotypic heterogeneity. Parallels may be drawn, in this respect, with the contribution of the *N0D2/CARD15* gene in CD.[36,37] The number of subjects with UC in the data sets studied was relatively small, and these may have been underpowered to identify a modest contribution. Moreover, the different ethnicity and study populations may confound the overall picture,[38] as also shown by the emerging data regarding *N0D2/CARD15* in European populations.

Our haplotype data provide further insight into the contribution of the *MDR1* gene in determining susceptibility and disease phenotype. By combining the 2 SNPs, the haplotype 3435T/G2677 was shown to confer an increased susceptibility to UC ($P = .03$; OR, 1.44; 95% CI, 1.03–1.99) whereas haplotype C3435/2677T appeared to protect against the development of UC ($P = .002$; OR, .35; 95% CI, .17–.69). The effect was most pronounced in patients who were homozygotes for both *MDR1* 3435 TT and 2677 GG genotype, but we note that the overall number of this group was very low (2.4%). The likelihood ratio for the haplotypic distribution in UC was also significantly different when compared with controls ($P = .0056$). Thus, it appears that these variants can alter the risk for developing UC in a bidirectional fashion. It is particularly interesting that the at-risk haplotype contains allelic variants associated with reduced P-glycoprotein expression in vivo, whereas the alleles on the protective haplotype have been associated with increased expression.

In recent years, the case for the involvement of the *MDR1* gene and P-glycoprotein 170 in determining susceptibility in IBD has become increasingly persuasive. It is clear, however, that the role of the *MDR1* gene/P-glycoprotein 170 in inflammation is likely to be more complex than originally believed. Most pertinently, there seems to be cell, tissue, and even regional organ-specific differences in the regulation of both the function and the expression of P-glycoprotein 170.[39–43] Increasingly, in vitro and ex vivo studies, including data from our unit involving the HLA-B27 transgenic mice, suggest that P-glycoprotein expression is in fact reduced in the presence of colonic inflammation.[44–46] This together with the findings by Langmann et al, showing down-regulation of *MDR1* (with other detoxification genes) in colonic tissue of patients with UC, puts forward a compelling argument for an influential role of P-glycoprotein in determining susceptibility to UC.[10] We hypothesize that low levels of P-glycoprotein 170 expression in the gastrointestinal colonic epithelium increases susceptibility and high levels are protective.

Based on our data, the C3435T but not G2677T SNP is primarily associated with UC. This leads to the question whether C3435T is the functional variant or in linkage with another variant. Given that we did not show an association with G2677T alone and that 2-locus haplotypes were not superior in determining risk, we clearly cannot ascribe the significant association seen with C3435T to be secondary to linkage with G2677T/A. The functional effect of the C3435T, a syn-

onymous SNP that does not involve amino acid change, nevertheless remains controversial. It remains possible that this silent SNP can affect P-glycoprotein 170 activity/expression through, for example, effects on messenger RNA stability or codon preference. However, it is pertinent that the correlation between C3435T SNP and P-glycoprotein 170 activity/expression does not seem consistent across ethnic groups. Studies in white populations have shown an association with *MDR1* 3435 TT genotype and decreased P-glycoprotein 170 activity/expression, but the reverse is true for studies involving Japanese populations.[47–49] Therefore, we propose that the argument that this SNP lies within tight linkage disequilibrium with another unidentified causal variant remains the most plausible explanation. While the hypothetical model suggested by Brant et al, that C3435T lies in linkage disequilibrium with an unknown polymorphism that controls expression and that the G2677T SNP directly affects the innate P-glycoprotein 170 activity, cannot be conclusively disproved, this seems less likely in light of our data.

Does *MDR1* have a role as a pharmacogenetic marker? Farrell et al suggested that high P-glycoprotein 170 expression was associated with failure of medical treatment in IBD.[11] The study did not investigate the genetic contribution of the *MDR1* gene. The stratification to severe disease and need for surgery in our study was originally driven by the hypothesis that the CC genotype (in which some studies have shown to be associated with high expression) can predict corticosteroid resistance (severe disease) and therefore surgery. This in fact was clearly shown not to be the case in our study, with the trend being completely reversed (*MDR1* 3435-TT genotype higher in both patients with severe disease and requiring surgery). Recent data from the Oxford data set in abstract form even suggest that the TT genotype of the *MDR1* 3435 SNP may in fact be useful to predict surgery in UC, in conjunction with other genetic markers.[50]

Are allelic variants of the *MDR1* gene implicated in CD? Overall, we detect no significant associations with CD and the subphenotypes according to the Vienna classification of age at onset, disease location, and behavior (data not shown). In particular, no association was seen when we specifically considered only Crohn's colitis with either of the 2 SNPs. We do note, however, trends of associations with 2-locus haplotype (haplotypes 3435T/2677T: OR, .85; $P = .18$; C3435/2677T: OR, 1.39; $P = .19$; and 3435T/G2677: OR, 1.29; $P = .16$). Our CD population is smaller, and given the heterogeneity involved in the presentation of CD, there may be statistical limitations in conclusively confirming or refuting the hypothesis of association with CD.

In conclusion, our study provides robust evidence to support a role of the *MDR1* gene in the pathogenesis of UC. Germline *MDR1* variation determines both disease susceptibility and course in the Scottish population. The data point to the presence of more than one functional variant or a more haplotype-specific effect and underline the need for parallel functional studies and haplotype analyses.[51]

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