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PHARMACOGENETICS

Possible role of *MDR1* two-locus genotypes for young-age onset ulcerative colitis but not Crohn's disease

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Abstract
Background The role of the single nucleotide polymorphisms (SNPs) on positions 2677G>T/A and 3435C>T of the multi-drug-resistance gene 1 (*MDR1*) in inflammatory bowel disease (IBD) remains unclear.
Aims To further elucidate the potential impact of *MDR1* two-locus genotypes on susceptibility to IBD and disease behaviour.
Patients and methods Three hundred eighty-eight German IBD patients [244 with Crohn's disease (CD), 144 with ulcerative colitis (UC)] and 1,005 German healthy controls were genotyped for the two *MDR1* SNPs on positions 2677G>T/A and 3435C>T. Genotype–phenotype analysis was performed with respect to disease susceptibility stratified by age at diagnosis as well as disease localisation and behaviour.
Results Genotype distribution did not differ between all UC or CD patients and controls. Between UC and CD patients, however, we observed a trend of different distribution of the combined genotypes derived from SNPs 2677 and 3435 (χ^2=15.997, *df*=8, *p*=0.054). In subgroup analysis, genotype frequencies between UC patients with early onset of disease and controls showed significant difference for combined positions 2677 and 3435 (χ^2=16.054, *df*=8, *p*=0.034 for age at diagnosis ≥25, lower quartile). Herein the rare genotype 2677GG/3435TT was more frequently observed (odds ratio=7.0, 95% confidence interval 2.5–19.7). In this group severe course of disease behaviour depended on the combined *MDR1* SNPs (χ^2=16.101, *df*=6, *p*=0.017 for age at diagnosis ≥25). No association of *MDR1* genotypes with disease subgroups in CD was observed.
Conclusions While overall genotype distribution did not differ, combined *MDR1* genotypes derived from positions 2677 and 3435 are possibly associated with young age onset of UC and severe course of disease in this patient group.

Keywords *MDR1* · P-glycoprotein · Inflammatory bowel disease · Crohn's disease · Ulcerative colitis

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Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBD) characterised by intermittent and recurrent inflammation of the intestinal mucosa. The increased risk for IBD in first-degree relatives as well as the high disease concordance in monozygotic twins emphasises the importance of a genetic influence for disease development [1]. As a result, a number of putative

susceptibility loci for IBD could be identified by genome-wide linkage analyses [2]. For alterations of the *CARD-15*, *OCTN1/2, DLG5,* and *NOD1* genes, genetic variability was shown to play a significant role in IBD disease susceptibility [3].

The multi-drug-resistance gene 1 *MDR1* (*ABCB1*) is located on chromosome 7q21.1 and maps to one of the IBD susceptibility loci. *MDR1* first drew attention as a possible candidate gene when it was shown that knockout mice deficient for the rodent homologue MDR-1a spontaneously developed UC-like colitis [4]. *MDR1* codes for a transmembrane protein, called P glycoprotein (P-gp), which functions as an efflux pump actively transporting endogenous and exogenous substrates out of cells [5]. P-gp is located in the epithelial surfaces of various organs such as the intestine, liver and kidney, as well as in the endothelial lining of the blood–testis and the blood–brain barrier. There, P-gp is thought to play an important role as part of a protective barrier in the absorption, distribution and excretion of xenobiotics. In the intestine, P-gp is mainly located in the apical surfaces of enterocytes, where its expression increases longitudinally, with lowest levels in the duodenum and highest levels in the colon [6, 7]. In human intestinal biopsies, there is a two- to eightfold variation in the expression of P-gp between individuals [8].

Several observational studies have reported associations between polymorphisms in the *MDR1* gene and susceptibility to IBD, though these results were not always consistent. Overrepresentations of the T allele and the TT genotype at position 3435 were observed in patients with UC but not CD in two studies [9, 10]. Whereas other investigators were not able to support these results for the SNP 3435 in UC patients [11–14], two recent meta-analyses confirmed the association of the 3435C>T single-nucleotide polymorphism (SNP) and UC [15, 16]. Similarly, the *MDR1* SNP 2677 was either associated with IBD development [17] or it was not [10].

As was shown for the example of genetic influence of *MDR1* polymorphisms on pharmacokinetic data of P-gp probe drugs, combined SNP analysis was able to explain some of the discrepancies of single-variant analyses [18, 19]. Haplotypes derived from different *MDR1* SNPs 2677, and 3435 as well as 1236, 2677 and 3435 were reported to be associated with either UC or UC and therapy-refractory CD, respectively [10, 20]. For the variants on positions 2677 and 3435, significant pairwise linkage disequilibrium has already been shown [18], which could lead to possible artefacts in single-variant analyses. Therefore, to unravel the inconsistent results, our exploratory study was based on genotypes that combine the two most interesting *MDR1* SNPs: 2677G>T/A and 3435C>T. In a case-control design, we studied the influence of this two-locus *MDR1* gene variability on susceptibility to IBD. In further genotype–phenotype analyses, we tested patient subgroups with respect to age at diagnosis and further phenotypic variables, such as disease severity and localisation and steroid response.

Material and methods

Patients and healthy controls

We included 388 patients in the study with diagnosed CD or UC, including clinical, endoscopic, radiological and histological findings according to standardised criteria [21]. Subjects with colitis indeterminata were excluded. All patients were recruited from the University Clinic Charité – Universitätsmedizin Berlin (Campus Mitte and Campus Virchow), a tertiary referral centre. Data were obtained through retrospective collection from patients' clinical charts. The control group consisted of 1,005 healthy unrelated volunteers (781 men and 224 women) from the Berlin area, with a median age of 29 (range, 18–68) years. Genotype data from 461 controls were published previously [22]. Patients and healthy controls were Caucasians. The study was approved by the ethics committee of the Charité – Universitätsmedizin Berlin, Campus Mitte, and informed consent was obtained from each study participant.

Phenotypic assessment

The following data of patients with UC and CD were obtained: gender, age at diagnosis, disease localisation and behaviour, type and date of surgery (e.g. ileocecal resection, small- or large-bowel resection, reoperation), response to glucocorticoid treatment and severe course of disease (Table 1).

The following definitions were taken into account: Disease localisation was defined as the maximum extent of digestive involvement at the latest follow-up. Information was obtained through endoscopic (including upper endoscopy, capsule endoscopy and colonoscopy), radiological [small-bowel X-ray or computerised tomography (CT) enteroclysm] or histological examination. In CD patients, disease localisation and behaviour were categorised according to the Vienna classification, as described elsewhere [23]. Stenotic and perforating disease was recorded according to criteria already published [24]. In UC disease, localisation and extent was defined as pancolitis with the disease extending beyond the splenic flexure, left-sided colitis as disease extending to the splenic flexure and proctitis with disease limited to the rectum.

In patients having received glucocorticoid treatment for IBD (n=115 for UC and n=186 for CD patients), steroid response was defined as follows: steroid responsiveness (tapering possible in the past after each flare), steroid

Table 1 Demographic and clinical characteristics of ulcerative colitis (UC) (n=144) and Crohn's disease (CD) (n=244) patients. All patients were Caucasian

Disease type, and patient and clinical characteristics	Numbers
Ulcerative colitis	
Gender (M/F)	66/78
Age at diagnosis (median age in years)	32
Interquartile (years)	25–46
Disease localisation	
Pancolitis + backwash ileitis	15 (10.4%)
Pancolitis	59 (41.0%)
Left-sided colitis	54 (37.5%)
Proctitis	8 (5.6%)
Unknown	8 (5.6%)
Steroid use in the past	116 (80.6%)
Steroid responsive	68 (58.6%)
Steroid dependent	21 (18.1%)
Steroid resistant	27 (23.3%)
Unknown	0
Severe course of disease[a]	41 (28.5%)
Extraintestinal manifestations	95 (66.0%)
Primary sclerosing cholangitis	13 (9.0%)
Crohn's disease	
Gender (M/F)	93/151
Age at diagnosis (median age in years)	28
Interquartile (years)	21–37
Age at diagnosis ≤40 (A1)[c]	194 (79.5%)
Disease localisation[c]	
Ileal (L1)	28 (11.5%)
Colonic (L2)	25 (10.2%)
Ileocolonic (L3)	112 (45.9%)
Upper GI (L4)	71 (29.1%)
Unknown	8 (3.3%)
Disease behaviour[c]	
Inflammatory (B1)	60 (24.6%)
Stricturing (B2)	72 (29.5%)
Penetrating (B3)	104 (42.6%)
Unknown	8 (3.3%)
Ileocecal resection	82 (33.6%)
Reresection	32 (13.1%)
Steroid use in the past	186 (76.2%)
Steroid responsive	117 (63.0%)
Steroid dependent	53 (28.5%)
Steroid resistant	13 (7.0%)
Unknown	3 (1.5%)
Severe course of disease[b]	70 (28.7%)
Extraintestinal manifestations	123 (50.4%)
Primary sclerosing cholangitis	6 (2.5%)

[a] Defined as continuous use of steroids for more than 1 year or treatment with i.v. cyclosporine or proctocolectomy due to failure of medical therapy

[b] Defined as continuous steroid use for more than 1 year or treatment with infliximab

[c] Age at diagnosis (A1–A2), disease localisation (L1–L4) and disease behaviour (B1–B3) defined according to the Vienna Classification of CD. [24]

Table 2 Allelic and genotype frequencies for Crohn's disease (CD) and ulcerative colitis (UC) patients and controls for *MDR1* single-nucleotide polymorphisms 2677G>T/A and 3435 C>T

cDNA position	Exon	State	Observed frequency					
			Allele	*n*	% (95% CI)	Genotype	*n*	% (95% CI)
2677	21	Cases CD	G	265	54.3 (48.8–59.7)	GG	68	27.9 (20.6–35.9)
			T	213	43.6 (38.2–49.0)	GT	122	50.0 (41.4–58.6)
			A	10	2.0 (0.9–4.1)	GA	7	2.9 (0.9–6.8)
						TA	3	1.2 (0.2–4.4)
						TT	44	18.0 (12.3–25.1)
		Cases UC	G	167	58.0 (51.0–65.0)	GG	52	36.1 (25.9–47.0)
			T	115	40.0 (33.0–47.0)	GT	60	41.7 (30.9–53.0)
			A	6	2.0 (0.7–4.9)	GA	3	2.1 (0.3–7.3)
						TA	3	2.1 (0.3–7.3)
						TT	26	18.1 (10.5–27.7)
		Controls	G	1130	56.2 (53.6–58.9)	GG	307	30.6 (26.8–34.5)
			T	840	41.8 (39.2–44.4)	GT	492	49.0 (44.8–53.2)
			A	40	2.0 (1.3–2.8)	GA	24	2.4 (1.4–3.9)
						TA	14	1.4 (0.6–2.6)
						TT	167	16.6 (13.6–19.9)
						AA	1	0.1 (0.0–0.7)
3435	26	Cases CD	C	220	45.1 (40.6–50.0)	CC	42	17.2 (11.8–23.5)
			T	268	54.9 (50.4–59.4)	CT	136	55.7 (47.9–63.2)
						TT	66	27.0 (20.5–34.2)
		Cases UC	C	148	51.4 (45.5–57.3)	CC	42	29.2 (20.5–38.8)
			T	140	48.6 (42.7–54.6)	CT	64	44.4 (34.5–54.8)
						TT	38	26.4 (18.1–35.8)
		Controls	C	941	46.8 (44.6–49.0)	CC	223	22.2 (19.1–25.5)
			T	1069	53.2 (51.0–55.4)	CT	495	49.3 (45.4–53.1)
						TT	287	28.6 (25.2–32.0)

dependence (at least 10 mg of prednisolone equivalent per day are necessary to preserve remission after two failed attempts of reduction) and steroid resistance (no remission obtainable by high-dose steroid therapy of 40–100 mg of prednisolone equivalent per day over 6 weeks). Severe course of disease was defined as continuous use of steroids for more than 1 year or treatment with i.v. cyclosporine or proctocolectomy due to failure of medical therapy in UC and continuous steroid use for more than 1 year or treatment with infliximab in CD.

Genotyping

Genomic DNA was obtained from peripheral blood using the DNeasy Tissue Kit (QIAGEN GmbH, Hilden, Germany). Genotyping of the tri-allelic SNP exon 21 position 2677G>T/A (rs2032582) and the biallelic SNP exon 26 position 3435C>T (rs1045642) was performed by polymerase chain reaction restriction fragment-length polymorphism (PCR-RFLP) analysis as well as real-time PCR assays in a LightCycler, as previously described [22, 25]. Positions of *MDR1* SNPs refer to the known *MDR1* complementary DNA (cDNA), with the first base of the ATG start codon set to 1 [26]. Genotyping for the three common *CARD15* mutations (Arg702Trp, Gly908Arg and Leu1007finsC) in IBD patients was carried out as described [24].

Haplotype analysis

The program HAP version 0.2.1 was used to calculate haplotype distribution and inferring haplotype pairs for the genotype samples of UC, CD and control individuals. HAP is a program that extends SNPHAP to multiallelic markers. The Hardy–Weinberg equilibrium of polymorphisms as well as the linkage disequilibrium between SNPs 2677G>T/A and 3435C>T and a chi-square test for significance were calculated with Genepob Web version 3.4. (http://wbiomed.curtin.edu.au/genepop/).

Statistical analysis

We studied in a case-control design the possible association between the two-locus *MDR1* genotypes and susceptibility to IBD (UC and CD). In addition, we performed genotype–phenotype analyses with respect to age at diagnosis and several phenotypic variables. Associations between categorical variables were tested with contingency table analysis using likelihood-ratio chi-square statistics (exact test if

Table 3 Frequencies of the combined genotypes of the *MDR1* single-nucleotide polymorphisms 2677G>T/A and 3435C>T in Crohn's disease (CD) and ulcerative colitis (UC) patients as well as in controls

Genotype 2677/3435	Observed frequencies					
	Cases (CD)		Cases (UC)		Controls	
	n	(%)	*n*	(%)	*n*	(%)
GG/CC	36	14.8	32	22.2	184	18.3
GG/CT	29	11.9	13	9.0	99	9.9
GG/TT	3	1.2	7	4.9	24	2.3
GT/CC	3	1.2	6	4.2	23	2.2
GT/CT	96	39.3	45	31.3	362	36.0
GT/TT	23	9.4	9	6.3	107	10.6
TT/CC	–	0.0	1	0.7	2	0.2
TT/CT	5	2.0	3	2.1	16	1.6
TT/TT	39	16.0	22	15.3	149	14.8
GA/CC	3	1.2	2	1.4	14	1.4
GA/CT	4	1.6	1	0.7	6	0.6
GA/TT	–	0.0	–	0.0	4	0.4
TA/CC	–	0.0	1	0.7	–	0.0
TA/CT	2	0.8	2	1.4	11	1.1
TA/TT	1	0.4	–	0.0	3	0.3
AA/CC	–	0.0	–	0.0	–	0.0
AA/CT	–	0.0	–	0.0	1	0.1
AA/TT	–	0.0	–	0.0	–	0.0
total	244		144		1005	

necessary). All procedures were performed with SPSS (version 12.0.1, SPSS Inc., Chicago, IL, USA), except for confidence-interval determination. Exact 95% confidence intervals were calculated by StatXact-5, version 5.0.3. $P<0.05$ was considered test-wise statistically significant. Because of the exploratory character of the study, we did not apply correction for multiple testing.

Results

Genetic variability in *MDR1* and general susceptibility analysis

Distributions of alleles and genotypes of *MDR1* SNPs 2677G>T/A and 3435C>T in UC and CD patients as well as in controls are listed in Table 2. Both *MDR1* polymorphisms conformed to Hardy–Weinberg equilibrium in each study group. For the triallelic SNP 2677, all different nucleotides were found. The nucleotide 2677A was observed with a low frequency of about 2%. Because of the rarity of this allele and similarities in its distribution between all study groups, the 2677A variant was excluded from further statistical analysis.

Between SNPs 2677G>TA and 3435C>T, we found a statistically significant linkage disequilibrium (LD) ($P=0.0001$). LD between these two variants could lead to possible artefacts in single-variant analyses and emphasises the need for a two-locus genotype approach. Therefore, frequencies of *MDR1* genotypes and corresponding haplotypes derived from combination of SNPs 2677G>T/A and 3435C>T in UC and CD patients and controls, respectively,

Table 4 Predicted frequencies of the haplotypes of the *MDR1* single-nucleotide polymorphisms 2677G>T/A and 3435C>T in Crohn's disease (CD) and ulcerative colitis (UC) patients as well as in controls

Haplotype	Predicted frequencies		
	Cases (CD)	Cases (UC)	Controls
	%	%	%
2677G/3435C	41.9	44.5	43.1
2677T/3435T	41.7	35.1	39.5
2677G/3435T	12.4	13.5	12.8
2677T/3435C	1.9	4.8	2.6
2677A/3435C	1.3	2.1	1.5
2677A/3435T	0.8	0.0	0.6

Table 5 Stratification of *MDR1* genotypes with respect to *CARD15* risk genotype[a]

MDR1	GG/CC	GG/CT	GG/TT	GT/CC	GT/CT	GT/TT	TT/CC	TT/CT	TT/CT
CARD15 negative									
Count	29	8	7	4	37	8	1	3	16
Expected count	26.2	10.6	5.7	4.9	36.8	7.4	0.9	2.7	18
CARD15 positive									
Count	3	5	0	2	8	1	0	0	6
Expected count	3.7	2.5	0.8	1.1	8.1	1.7	0.2	0.6	3.9

[a] *CARD15* risk genotype (*CARD15* positive) defined as carrying at least one risk variant (Arg702Trp, Gly908Arg, Leu1007finsC)

were analysed. As shown in Table 3, 16 of 18 possible two-locus genotypes were found. For individuals homozygous at both SNPs or heterozygous at only one position, the haplotype could be assigned unambiguously. For the genotype 2677GT/3435CT, two haplotypes are possible, but the haplotype pair GCxTT was calculated to be more probable (Table 4).

We found no differences in genotype distributions of gender (χ^2=9.756, df=8, P=0.31). Therefore, a common analysis including men and women was executed. No significant difference in distributions of two-locus genotypes and haplotypes derived from both SNP positions were observed between controls and UC (χ^2=9.601, df=8, P=0.294 for genotypes) or CD patients (χ^2=6.970, df=8, P=0.565 for genotypes). In order to determine genetic interactions between *CARD15*- and *MDR1* genotype, data of CD patients were stratified for *MDR1* genotypes with respect to the *CARD15* genotype. We defined a *CARD15* risk genotype as carrying at least one allele within one of the three common *CARD15* variants (Arg702Trp, Gly908Arg, Leu1007finsC). However, *CARD15* genotypes were randomly distributed with respect to *MDR1* genotypes. Therefore, hidden genetic effects of *CARD15* are unlikely (see Table 5).

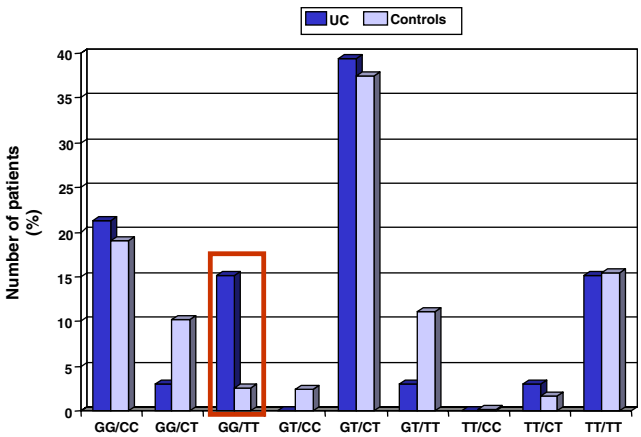

Fig. 1 *MDR1* genotype distribution for ulcerative colitis (UC) ≤25 at age at diagnosis and controls. Significant difference of *MDR1* genotype distribution of combined positions 2677 and 3435 between UC patients ≤25 at age at diagnosis (n=33) and controls (n=966, P=0.034). The rare 2677A variant was excluded from this analysis. The rare genotype GG/TT was overrepresented in the patient group (framed in *red*)

Association between *MDR1* genotypes and disease status

UC and CD are often subject to combined analysis, assuming that there are similarities in the aetiology of both chronic IBDs. In our patients, however, a trend to different genotype distributions of UC and CD patients was found (χ^2=15.997, df=8, P=0.054). Because this result pointed to a varying influence of *MDR1* genotypes between UC and CD, we refrained from analysing the IBD group as a whole.

MDR1 genotypes and UC phenotype

Due to varying incidences of UC in different age groups [27] and a probably varying age at diagnosis in case of a different genetic disposition, we evaluated the groups of younger and older patients separately using 25% of patients with youngest and oldest age at diagnosis. After stratification, we found a significant difference in two-locus genotype distribution in UC patients with an age at diagnosis ≤25 (lower quartile) compared with controls (χ^2=16.054, df=8, P=0.034, Fig. 1). Especially, the rare genotype 2677GG/3435TT appears to be overrepresented in UC patients [five out of 33 patients, odds ratio (OR)=7.0; 95% CI: 2.5–19.7; (Fig. 1)]. Analysing younger patients made the association even stronger, which further corroborates our result (e.g. age at diagnosis ≤22, χ^2=21.027, df=8, P=0.004). In the patient group with an age at diagnosis ≥46 years (upper quartile), no differences to the control group were found.

No differences in two-locus *MDR1* genotype and haplotype distributions were observed between cases and controls when UC patients were stratified for disease localisation (χ^2=13.585, df=21, P=0.952 for genotypes), steroid response (χ^2=10.834, df=16, P=0.922 for genotypes) and severe course of disease behaviour (χ^2=12.530, df=8, P=0.120 for genotypes). However, subgroup analysis for severe course of disease behaviour stratified for age at

diagnosis revealed significant differences in genotype distribution between patients with and without a severe course of disease also only in the group of younger UC patients (χ^2=16.101, df=6, P=0.014; age at diagnosis ≤25, lower quartile), indicating a possible different genetic influence of the *MDR1* gene for these two different phenotypes in younger patients.

MDR1 genotypes and CD phenotype

In CD, stratification for disease localisation (χ^2=13.585, df=21,P=0.949 for genotypes), severe course of disease behaviour (χ^2= 3.687, df=7, P=0.889 for genotypes), ileocecal resection (χ^2=12.319, df=7, P=0.129 for genotypes) or steroid response (χ^2=12.844, df=14, P=0.597 for genotypes) did not reveal differences in the distribution of *MDR1* genotypes and haplotypes when compared with controls. In accordance with UC, a subgroup analysis of CD patients with young and old age at diagnosis was performed separately. However, no significant differences in *MDR1* genotype distribution between patients of younger or older age at diagnosis (lower and upper quartile, respectively) and controls could be observed.

Discussion

UC and CD are often considered as one entity, a notion that mainly relies on uncertainty about the underlying pathophysiological changes in both IBDs [1]. However, next to differences in clinical manifestations, histological findings and therapy regimens, variations in genes such as *CARD15* have been identified, indicating that CD and UC aetiologies may not be interchangeable [3]. With respect to *MDR1* variants 2677G>T and 3435C>T, our results of a trend to different genotype distributions between UC and CD patients provide a further hint of a genetic difference between both IBDs. This is in line with studies reporting a lower-intestinal expression of messenger RNA of *MDR1* in UC than in CD patients and findings of reduced transcription of *MDR1* by the pregnane X-receptor (PXR) in UC but not CD [28, 29].

We found no general genotype or haplotype association with UC or CD, respectively, when compared with healthy controls. This lack of a general association is in accordance with results of several other studies [10–14]. The primary positive report by Schwab et al. of an association of the 3435 T allele and TT genotype with susceptibility to UC in a comparable German population has been challenged by some authors for its study design [11, 13]. Compared with our data, the allele frequencies for the SNP 3435 of their control group were out of our 95% CI, a possible explanation for the conflicting results [9].

In subgroup analysis, stratification for age at diagnosis, however, revealed a relevant influence of two-locus *MDR1* genotypes for young UC patients on susceptibility and severity of disease. Five of 33 individuals in our UC patient subgroup with an age at diagnosis ≤25 (lower quartile)—and even more interesting, five of 23 patients in the group with an age at diagnosis <22—carried the rare genotype 2677GG/3435TT, which in a study by Ho et al. had already been associated with an increased risk for UC [10]. The close relationship between two-locus *MDR1* genotypes and early onset of UC is interesting, as early onset disease may have a stronger familial and consequentially genetic contribution [31]. In their study, Ho et al. did not carry out a separate analysis regarding age at disease onset [10] but, similar to our study, their UC cohort comprised patients with a median age at UC onset of 35 years, including even a relatively high number of 10% of patients with an age at diagnosis younger than 16 years. Therefore, it can be speculated that phenotype–genotype results evaluating associations between UC and *MDR1* depend on the relative proportion of patients with early disease onset. Other studies, which could not find associations between genetic variability in *MDR1* and susceptibility to UC, did not provide or account for age-related data [11–14]. In contrast to our data, a single variant analysis with an age-at-onset stratified approach in a small Japanese cohort of 66 UC patients showed an association of the T allele and the TT genotype on position 3435 of the *MDR1* gene with UC in their subgroup of older UC patients [32]. However, as frequencies of the 3435 T allele and TT genotype vary substantially among populations of different ethnic origin [33], the results of this Japanese study further underline the need to include age at disease onset in future association analyses.

Another finding of our study was the absence of associations between *MDR1* genotypes and CD. This result is in agreement with most other observational studies [9, 11, 30]. Similar to Schwab et al [9] and Croucher et al [11], we were also unable to find relations between *MDR1* and *CARD15* variants in CD patients. On the other hand, Potocnik et al. reported an association between *MDR1* genotypes and patients with refractory CD who did not respond to standard therapy [20]. Our results did not reveal differences in therapeutic response to steroid treatment in either patient group. However, steroids are not only known to be substrates of P-gp, they are also known to be involved in the regulation of *MDR1* transcription [34]. These changes in the expression of P-gp by steroid treatment may have covered influences of *MDR1* genotypes on therapy response and dosing regimens in our patients.

Recent in vitro studies have revealed the possible functional impact of single *MDR1* SNPs 2677 and 3435 and their combinations on P-gp activity and expression

[35–37]. The new information is of special interest because animal data indicated that susceptibility to UC development may be influenced by alterations in P-gp activity. Knockout mice lacking *Mdr1*a showed increased susceptibility for colitis, even in the absence of immune dysfunction or pathogen stimulus [4]. Furthermore, lack of *Mdr1*a activity results in dysfunction of the intestinal barrier and an increase of bacterial translocation in mice [38]. Therefore, it may be speculated that *MDR1* genotypes altering the activity of P-gp will lead to barrier dysfunctions and intestinal inflammation and ultimately will give rise to the development of UC in humans.

In conclusion, our study could not reveal differences in *MDR1* genotypes between controls and patients suffering either from UC or CD. However, our data indicate that two-locus *MDR1* genotypes derived from positions 2677 and 3435 may be associated with young age at UC onset and possibly severe course of disease in this group of young patients. We emphasise the need for multilocus genotype analysis and, in future studies, would propose to give special regard to patients with genotype 2677GG/3435TT to possibly confirm this *MDR1* genotype as a potential risk factor for the (early) development of UC. As no correction for multiple testing was applied, our results should be regarded as exploratory and awaiting further confirmation by other studies, preferably with children with IBD.

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