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Lack of Association Between the C3435T *MDR1* Gene Polymorphism and Inflammatory Bowel Disease in Two Independent Northern European Populations

Dear Sir:

Recently, Schwab et al.[1], writing in GASTROENTEROLOGY, reported an association between the silent C3435T polymorphism in exon 26 of the human multidrug resistance 1 (*MDR1*) gene and ulcerative colitis (UC). Their study, which indicated that the T-allele and the TT-genotype were overrepresented in UC patients, consisted of a gender-balanced case-control analysis of 275 inflammatory bowel disease (IBD) patients (149 UC, 126 Crohn's disease [CD]) plus 998 healthy controls. The gene product of *MDR1*, P-glycoprotein, has been proposed to act as an important barrier against xenobiotics in intestinal epithelial cells. As Schwab et al. point out, much of the evidence for this has come from experimental models of IBD in mice that are *MDR1* deficient.[1–3] The *CARD15* gene product appears to play a role in bacterial lipopolysaccharide signature recognition and subsequent mediation of the immune response and recently coding variants in the *CARD15* gene have been associated with Crohn's disease.[4–6] The suggestion that *MDR1*, another gene with a role in protecting the intestinal epithelia from bacterial invasion, may play a role in UC susceptibility is therefore of great interest.

We have therefore attempted to critically replicate the findings of Schwab et al.[1] by genotyping the C3435T *MDR1* polymorphism (rs1045642; Assay-on-Demand (Applied Biosystems) reference: c_7586657_1) in 2 well established and independent northern European cohorts: (1) a German cohort consisting of 171 multiplex families (containing 262 CD probands and 134 UC probands) plus 553 trios (367 CD, 186 UC) and 531 normal German controls; (2) a UK cohort consisting of 93 multiplex families (containing 164 CD and 87 UC probands) plus 164 normal UK controls. These cohorts and their ascertainment criteria have been described in detail previously.[6] We have subjected these cohorts to case-control analysis by randomly extracting a single affected individual from each pedigree and then randomly extracting controls so as to create a gender-balanced case-control set for each analysis. The significance of an initial χ^2 statistic was evaluated by permuting the case and control data 1000 times. Odds ratios were calculated and their confidence intervals estimated by through 1000 bootstrap replicates. Additionally, transmission disequilibrium tests (TDT) were performed on the pedigree data using TRANSMIT with the robust variance estimator option. Significances were verified with 10000 bootstrap replicates. Analyses were carried out using the standard diagnostic categories IBD, CD, and UC. Summary statistics are given in Table 1 (more detailed supplementary statistics are available on request from the authors).

No evidence of association was detected between the C3435T *MDR1* polymorphism and the IBD, CD, or UC phenotypes in any analysis. Furthermore, case-control analysis without gender balancing did not lead to significant association (for the German cohort, UC: allelic OR = 0.99 [0.80–1.22], $P = 0.89$; genotypic OR = 0.95 [0.62–1.46], $P = 0.83$. CD: allelic OR = 1.01 [0.85–1.20], $P = 0.92$; genotypic OR = 1.01 [0.70–1.45], $P = 0.94$). Relaxation of the gender-matching criterion in the Schwab et al. paper led to a much stronger UC association. To rule out any possible interaction with the *CARD15* gene, the larger German cohort was stratified for individuals carrying one or more of the 3 CD predisposing variants (*CARD15*+) and for those that were wild-type for this gene (*CARD15*−). Again, no association was detected for any phenotype (TDT results, UC: *CARD15*+, $\chi^2 = 0.20$, $P = 0.65$; *CARD15*−, $\chi^2 = 0.03$, $P = 0.87$. CD: *CARD15*+, $\chi^2 = 0.01$, $P = 0.90$; *CARD15*−, $\chi^2 = 0.46$, $P = 0.49$).

Table 1. Summary Association Statistics for the C3435T *MDR1* Polymorphism in the German and British Cohorts

	Case-control			Transmission
Population	n[a] cases/controls	Allele OR[b] (95% CI)	Genotype OR[b] (95% CI)	TDT O/E[c], χ^2, *P* value
German				
IBD	718/404	1.00 (0.86–1.14)	1.01 (0.75–1.33)	957/960.35, 0.05, $P = 0.82$
CD	494/397	1.00 (0.86–1.17)	1.02 (0.73–1.41)	634/638.36, 0.14, $P = 0.71$
UC	263/424	0.98 (0.83–1.17)	0.97 (0.68–1.43)	323/321.99, 0.01, $P = 0.90$
British				
IBD	90/122	1.24 (0.87–1.71)	1.40 (0.75–2.61)	246/248.23, 0.13, $P = 0.72$
CD	68/112	1.23 (0.85–1.81)	1.40 (0.71–2.85)	158/162.01, 0.63, $P = 0.43$
UC	44/114	1.08 (0.71–1.68)	1.14 (0.52–2.77)	88/86.21, 0.21, $P = 0.62$

[a]Number of controls after gender-ratio balancing.
[b]Odds ratio and 95% confidence interval. Allelic: C vs. T; genotypic CC vs. TT.
[c]Observed/expected transmissions of the T-allele as calculated by TRANSMIT.

Our data clearly do not support the hypothesis that there is an association between UC (or CD) and the C3435T *MDR1* polymorphism. Although Schwab et al. took stringent precautions to limit the likelihood of a chance finding (for example, gender matching and checking for differences in allele frequency between different age groups) their result still depended entirely on a case-control analysis of only 149 UC patients for a mutation of apparently weak effect (genotype OR = 2.03 [1.04–3.95], $P = 0.045$).[1] Such a scenario is typical of association studies on complex diseases which have an inherent lack of power at anything less than very large sample sizes. Replication is therefore essential. We have attempted to verify this finding in 2 independent European cohorts using both a case-control strategy, analogous to Schwab et al., and a TDT based analysis (which is more resilient to latent effects such as population stratification). We have been unable to detect association. We look forward to further analyses of the possible role of the *MDR1* locus in IBD. However, our data suggest that it is doubtful that this locus is indeed associated with UC or CD.

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Reply. We read with interest the letter by Croucher et al. who were unable to confirm the reported association between the C3435T *MDR1* gene polymorphism and ulcerative colitis (UC) in a German and a British cohort population. We agree with the authors that association studies have many potential problems which have been discussed in detail elsewhere.[1,2] As recognized by Croucher et al., we had applied appropriate precautions in our case-control study to avoid these pitfalls by strictly following the guidelines to authors of genetic association studies by Bird et al.[3] Thus, a sex-matched study design was used, the age of cases/controls was comparable, all subjects were hospital-based, unrelated individuals with the same ethnicity (of white origin) and the phenotype of all patients (e.g., disease localization, immunosuppression) was described in detail.

When applying the same guidelines to the study by Croucher et al., we are concerned that there may have been confounding in various ways. Thus, although the authors mention that both IBD cohorts from Germany and the UK have been described in detail previously.[4] We were unable to extract important data. For example, the ethnic origin of their patients has not been stated. Since the frequencies of allele and genotype distribution for the *MDR1* polymorphism C3435T is highly variable between ethnic groups,[5,6] the possibility of major confounding should be kept in mind given that only 44 British UC cases were analyzed. Moreover, although only 93 British multiplex families (containing 164 CD patients and 87 UC patients) were included, the results from 68 CD and 44 UC patients (= 112 patients) were shown. Therefore, it is unclear whether indeed only a single affected individual from each pedigree was used in their analysis.

Secondly, association studies rely heavily on the accuracy of phenotype description.[2,3] Although we did not find *MDR1* genotype-dependent differences with respect to UC disease localization in our study (proctitis [11%], left-sided colitis [46%], pancolitis [43%]),[7] it cannot be ruled out that such differences may be present by a different composition of phenotypes. Unfortunately, Croucher et al. do not provide any data with regard to UC disease phenotype.

When we genotyped all our CD (n = 126) and UC patients (n = 149) for CARD15 mutations, the allele frequencies and the genotype distribution were significantly higher in CD as compared with UC patients, which is in keeping with the literature. Thus, a chance finding in our cohort with respect to the association of the C3435T polymorphism and UC became unlikely. Our data did not support the existence of a linkage between CARD15 genotypes (SNP 8, 12, and 13) and the C3435T polymorphism,[7] an observation now confirmed by Croucher at al.

In addition, McGovern et al. recently investigated cytochrome P450 3A5 and MDR1 polymorphisms as predictors for the need of colectomy in patients with UC.[8] Interestingly, the frequency of *MDR1* 3435TT-carriers in non-colectomy patients with pancolitis UC was 31.3%. Although neither CD patients nor healthy controls were investigated, this result is in keeping with the frequency of the 3435TT genotype in our UC study population (30.9%), which is also higher than in healthy subjects from several other European populations.[9]

In summary, we are not convinced with the argument of Croucher et al. that their data "clearly" do not support the hypothesis of an association between UC and the C3435T *MDR1* polymorphism. We agree, however, in seeing the need for more studies on the *MDR1* gene and intestinal P-glycoprotein expression as a possible determinant in gastrointestinal diseases.

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