ABCB1 3435C>T and 2677G>T/A polymorphisms in Polish and Bosnian patients with Crohn's disease – A preliminary report

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ABSTRACT

The role of *ABCB1* single nucleotide polymorphisms (SNPs) in the development of Crohn's disease (CD) remains unclear. Due to inconsistent results of several European population-based studies and limited information on populations from Poland and Bosnia and Herzegovina (B&H), we conducted a preliminary association study of two main *ABCB1* SNPs and CD. *ABCB1* 3435C>T and 2677G>T/A SNPs were analyzed in Polish and Bosnian patients with CD (n = 85 and n = 30, respectively) and controls (n = 82 and n = 30, respectively) using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for 3435C>T and allele-specific PCR for 2677G>A/T SNP. A deviation from Hardy-Weinberg equilibrium was found for both SNPs in Polish patients with CD, and for 2677G>A/T in Polish control group. The allele and genotype frequencies of the two *ABCB1* SNPs were not significantly different between the CD patients and controls in both populations ($p > 0.05$). Similarly, the genotype distribution of 3435C>T and 2677G>T/A SNPs was not significantly different between Polish and Bosnian patients with CD ($p > 0.05$). At least one mutated *ABCB1* allele was carried by 97.7% of Polish and 90.0% of Bosnian patients with CD. No association was found between the *ABCB1* SNPs and CD in the two populations. In conclusion, the two *ABCB1* SNPs may not contribute to CD susceptibility in the populations of Poland and B&H. Further studies with larger samples in both populations are warranted.

KEY WORDS: *ABCB1*; Crohn's disease; allele frequency; Poland; Bosnia and Herzegovina; 3435C>T; 2677G>T/A; single nucleotide polymorphism; SNP

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INTRODUCTION

Crohn's disease (CD) is a heritable complex disease characterized by chronic inflammation of the gastrointestinal tract. Together with ulcerative colitis, CD belongs to a group of intestinal disorders called inflammatory bowel disease (IBD) [1]. The common symptoms of CD include abdominal pain, fever, and other clinical signs of bowel obstruction, as well as diarrhea with blood and/or mucus in stool [1].

The etiopathogenesis of CD still remains unclear. However, familial aggregation as well as a higher concordance rate in monozygotic than in dizygotic twins, suggest the contribution of genetic factors to the development of CD [2]. Recent genome-wide association studies (GWAS) have successfully detected more than 160 IBD susceptibility loci, whereas 30 loci were unique to CD [3]. One of the key genes that might be involved in the pathogenesis of CD is ATP-binding cassette subfamily B member 1 (*ABCB1*); the protein is also known as multidrug resistance protein 1 or permeability glycoprotein 1 (P-gp) [4].

P-gp is an ABC transporter that regulates the passage of endogenous and exogenous substances through the cell membrane. P-gp is expressed in intestinal epithelial cells (IECs) and acts as a protective barrier against bacterial toxins, drugs and other xenobiotics in the gastrointestinal tract [4,5]. The *ABCB1* gene is located within 7q21.1, a chromosomal region suggested to be linked to IBD [5]. Moreover, among the most important polymorphisms linked to CD are 2677G>A/T (rs2032582) and 3435C>T (rs1045642) single-nucleotide

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polymorphisms (SNPs) located in the exons 21 and 26 of the *ABCB1* gene, respectively [5,6]. To date, contradictory results have been reported on the effect of 2677G>A/T SNP on P-gp expression [6]. Nevertheless, the presence of the variant allele might alter the P-gp affinity or stability for the substrate [6]. Moreover, the altered function of P-gp has also been associated with 3435C>T SNP of the *ABCB1* gene [5]. Although 3435C>T is a synonymous SNP, and the risk allele does not directly affect the P-gp amino acid sequence, it moderately alters mRNA stability of the *ABCB1* gene [6].

The aim of our study was to investigate the association of *ABCB1* 2677G>A/T and 3435C>T SNPs with CD in patients and controls from Poland and Bosnia and Herzegovina (B&H).

MATERIALS AND METHODS

Participants

The Local Bioethical Committees approved the protocol of this study (decision reference numbers 29-BS-4329/11 and KB-0012/183/13 from B&H and Poland, respectively). All participants provided written informed consent prior to the study enrollment. In this preliminary study, we collected blood samples from 227 participants (combined from both countries) for genotyping the two *ABCB1* SNPs. The samples from patients and controls from both populations are still being collected for the future research purposes. The characteristics of the Polish and Bosnian patients with CD and controls are shown in Table 1. A total of 85 Polish patients with CD and 82 controls and 30 Bosnian patients with CD and 30 controls were selected consecutively from a pool of patients undergoing diagnostic evaluation or treatment at the participating institutions, between January and July 2013. CD diagnosis was determined according to the clinical, radiological, endoscopic, and histological criteria [7]. The patients enrolled as controls had undergone diagnostic testing for reasons other than IBD and had a colonoscopy performed during their diagnostic evaluation, which excluded the possibility of undiagnosed CD. Age at the time of diagnosis of CD was obtained from a direct interview with patients or from their medical records.

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the Qiagen extraction kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The genotyping of *ABCB1* 3435C>T (rs1045642) was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, according to the previously described protocols [8]. *ABCB1* 2677G>A/T (rs2032582) was genotyped using allele-specific PCR (ASPCR), according to the method described by Kurzawski et al. [9]. The forward and reverse primers, as well as restriction enzymes and restriction recognition sites are shown in Table 2. DNA amplification was performed using a LabCycler device (SensoQuest GmbH, Göttingen, Germany). Genotypes were determined by electrophoresis of the restricted DNA fragments and allele-specific amplicons, in 2% agarose gel (Sigma-Aldrich Chemie Gmbh, Munich, Germany) stained with DNA-star dye (Lonza Inc., Rockland, ME, USA).

Statistical analysis

Data were analyzed using the GraphPad Prism v5.03 software (GraphPad Software Inc., San Diego, CA, USA). Genotype and allele frequencies were determined by direct counting. The SNPs were tested for the Hardy–Weinberg equilibrium (HWE) by comparing the observed genotype distributions with the expected values, using a χ^2 test. The differences in the genotype and allele distribution between CD patients and controls, in both populations, were tested using a χ^2 test with Yates' correction, where appropriate, or Fisher's exact test. All p values were two-sided and the statistical significance was set at $p < 0.05$.

RESULTS

Deviation from the HWE was found in the group of Polish patients with CD for both *ABCB1* SNPs, and in the Polish control group for 2677G>A/T SNP. The distribution of *ABCB1* genotypes and alleles in the two populations is shown in Table 3. In the Polish group, at least one mutated allele of *ABCB1* SNPs was found in 97.7% (n = 83) of CD patients and in 84.2% (n = 69) of controls. Comparably, in the Bosnian group, at least one mutated *ABCB1* allele was found in 90.0% (n = 27) of CD patients and in 90.0% (n = 27) of controls ($p > 0.05$). The allele and genotype frequencies of the two *ABCB1* SNPs were not significantly different between the CD patients and controls in both populations ($p > 0.05$). Similarly, the genotype distribution of 3435C>T and 2677G>T/A SNPs was not significantly different between Polish and Bosnian patients with CD ($p > 0.05$).

DISCUSSION

Recently, an increasing incidence of IBD has been reported, including CD and UC. It has been suggested that the susceptibility to IBD as well as individual response to therapy may be linked to genetic factors, such as *ABCB1* gene mutations. Here we investigated the association of two *ABCB1* SNPs with CD in populations from Poland and B&H. At least one mutated allele of 3435C>T or 2677G>T/A SNPs was carried by 97.7% of Polish and 90.0% of Bosnian

TABLE 1. Characteristics of cohorts from Poland and Bosnia and Herzegovina

Total, n=227	Poland		Bosnia and Herzegovina	
	CD patients, n=85	Controls, n=82	CD patients, n=30	Controls, n=30
Male, n (%)	48 (56.5)	44 (53.7)	15 (50)	14 (46.7)
Female, n (%)	37 (43.5)	38 (46.3)	15 (50)	16 (53.3)
Age at diagnosis (mean±SD)	33.9±12.9	35.5±21.8	44.1±14.5	61.3±15.2

SD: Standard deviation; CD: Crohn's disease

TABLE 2. Forward and reverse primers, restriction enzymes and restriction recognition sites for *ABCB1* polymorphisms

Gene, rs	Primers sequences	Restriction enzyme	Wild-type allele	Mutated allele
ABCB1				
rs1045642	5'-TGTTTTCAGCTGCTTGATGG-3' 5'-AAGGCATGTATGTTGGCCTC-3'	*Sau*3aI	158, 39 bp	197 bp
rs2032582	Forward sequence - Specific primers: allele A: 5'-TGAAAGATAAGAAAGAACTAGAAGGTA-3' Allele G: 5'-TGAAAGATAAGAAAGAACTAGAAGGTG-3' Allele T: 5'-TGAAAGATAAGAAAGAACTAGAAGGTT-3' Forward internal control: 5'-AGCAAATCTTGGGACAGGAA-3' Common reverse primer: 5'-AGTCCAAGAACTGGCTTTGC-3'		Control DNA fragment: 353 bp Allele-specific fragments: 222 bp	

TABLE 3. Genotype and allele distribution of *ABCB1* polymorphisms in patients with CD and controls

Gene polymorphism	Poland		Bosnia and Herzegovina	
	CD patients, n=85	Controls, n=82	CD patients, n=30	Controls, n=30
ABCB1 3435C>T (rs1045642)				
Genotypes, n (%)				
CC	12 (14.1)	17 (20.7)	4 (13.3)	7 (23.3)
CT	51 (60.0)	46 (56.1)	14 (46.7)	14 (46.7)
TT	22 (25.9)	19 (23.2)	12 (40.0)	9 (30.0)
Alleles, n (%)				
C	75 (44.1)	80 (48.8)	22 (36.7)	28 (46.7)
T	95 (55.9)	84 (51.2)	38 (63.3)	32 (53.3)
ABCB1 2677G>A/T (rs2032582)				
Genotypes, n (%)				
GG	13 (15.3)	21 (25.6)	4 (13.3)	6 (20.0)
GA	5 (5.9)	4 (4.9)	0	1 (3.3)
GT	50 (58.8)	47 (57.3)	18 (60.0)	16 (53.3)
AT	4 (4.7)	2 (2.4)	1 (3.3)	0
TT	13 (15.3)	8 (9.8)	7 (23.3)	7 (23.3)
Alleles, n (%)				
G	81 (47.7)	93 (56.7)	26 (43.3)	29 (48.3)
A	9 (5.3)	6 (3.7)	1 (1.7)	1 (1.7)
T	80 (47.1)	65 (39.6)	33 (55.0)	30 (50.0)
A or T	89 (52.4)	71 (43.3)	34 (56.7)	31 (51.7)

CD: Crohn's disease

patients with CD. The frequency of mutated alleles reported in our study is higher compared to the frequency described for a German cohort of patients with CD (85.2%) [10]. In our study, the frequency of 3435C>T mutated allele was 55.9% in the group of Polish patients with CD. This result for the 3435T allele is comparable to the results reported by the following studies: 57.4% in another group of Polish patients with CD [5]; 53.2% [10] and 54.9% [11] in German cohorts of CD patients; 53.0% in a Scottish [12]; 51.9% in British [13]; and 56.6% in New Zeland population [14]. Lower frequencies of the 3435T allele compared to our Polish group of CD patients were revealed in the next studies: 48.9% in another group of Polish patients with CD [15]; 40.9% in a Dutch group [16]; and 39% in an Italian group [17]. The frequency of 2677G>T/A mutated alleles (T or A allele, 52.4%) in our group of Polish patients with CD was higher than reported for the Scottish, 47.2% for 2677T [12]; British, 42.2% [13]; German, 45.6% [10] and New Zeland population, 46.4% [14]; while it was nearly identical to the 2677G>T/A frequency observed in the Italian population, 52% [17]. In our group of CD patients from B&H, the frequencies of 3435C>T and 2677G>T/A mutated alleles were 63.3% and 56.7% (T or A allele), respectively. These frequencies are

higher than those described for CD patients from southeastern and central European countries, including Slovenia (51.9% and 40.5%, respectively), Croatia (47.5% and 38.4% for 2677T, respectively), Greece (51.9% for 3435T), and Hungary (50.2% and 41.3%, respectively) [6,18-20].

To date, contradictory results have been reported on the association between *ABCB1* 3435C>T and 2677G>T/A variants and CD in European populations. According to Urcelay et al. [21], the *ABCB1* 3435CC genotype was significantly more frequent in Spanish patients with CD than in controls, and the carriage of the 3435C allele was significantly associated with CD [21]. Lal et al. [22] observed that the 3435T allele was significantly more frequent in Canadian patients with CD than in controls [22]. Although Huebner et al. [14] did not show any association of *ABCB1* 2677G>A/T or 3435C>T with CD in New Zealand patients with self-reported European ancestry, they indicated a significant association of *ABCB1* 3435T with an increased probability of ileal/stricturing phenotypes of CD [14]. Brinar et al. [6] showed no significant differences in the allele frequencies of both SNPs and the genotype frequencies of 2677G>T/A between Croatian patients with CD and controls, however, they suggested the protective role of heterozygous 3435CT genotype, as it was significantly less frequent in their CD patients [6]. In the other Polish population, Jazwinska-Tarnawska et al. [15] found a statistically significant correlation between gender and the 3435C>T genotype in CD and IBD patients, with a higher frequency of the 3435CT genotype among the males with CD and IBD compared to the females [15]. Similar to our results, other studies that reported no association between CD and the two *ABCB1* SNPs are as follows: in the other group from Poland [5]; in several groups from Germany for 3435C>T alleles and genotypes [10,11,23]; in German and British groups for 3435C>T allele [24]; and in a Greek population [19].

In a large, multicenter North American cohort Brant et al. [25] showed no association of *ABCB1* 2677G>A/T and 3435C>T with CD, however, the authors found an association between the 2677G allele and IBD [25]. In the Scottish and British populations there was also no association between the *ABCB1* SNPs and CD [12,13], but the frequency of 2677TT genotype was significantly increased in the British patients without the ileal CD phenotype [13]. Comparably, no association between the two *ABCB1* SNPs and CD was reported for the populations from the Netherlands, Hungary, and Italy [16,17,20], although there was a tendency for the 2677G allele to be associated with the disease susceptibility among Hungarian patients with CD [20]. Finally, two meta-analyses, the first including 9 [13] and the second including 13 case-control studies [26], revealed no association between the 3435T and 2677T [13] and 3435C>T [26] alleles, respectively, with CD. Despite the fact that most of the above-mentioned studies were carried out on rather small groups of participants, it is supposed that *ABCB1* 2677G>A/T and 3435C>T SNPs are not associated with CD in the European populations.

CONCLUSION

The current study is the first report on *ABCB1* 2677G>A/T SNP frequency in Polish patients with CD and *ABCB1* 3435C>T and 2677G>A/T SNP frequencies in Bosnian patients with CD. Our results indicate that the *ABCB1* SNPs do not contribute to CD risk in the populations from Poland and B&H. Overall, these data confirm the genetic complexity of CD. Nevertheless, due to the small sample size of the investigated cohorts, we plan to re-evaluate these results in subsequent studies with larger cohorts of CD patients from both populations.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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