Investigation of the functional single-nucleotide polymorphisms in the *BCRP* transporter and susceptibility to colorectal cancer

FATIH M. SARI[1], HAKAN T. YANAR[2] and GUL OZHAN[1]

[1]Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Istanbul 34116;
[2]Department of General Surgery, Faculty of Medicine, Istanbul University, Istanbul 34390, Turkey

Received October 14, 2014; Accepted October 21, 2014

DOI: 10.3892/br.2014.383

Abstract. Breast cancer resistance protein (*BCRP*) protects tissues by actively transporting xenobiotics and their metabolites out of the cells. *BCRP* is expressed in the apical membrane of normal intestinal and colonic epithelium. The *BCRP* substrates include a number of structurally unrelated compounds, such as drugs, pesticides, carcinogens and endogenous compounds. Although the functional and common *BCRP* alleles, 34G>A and 421C>A, are shown to vary by ethnicity, their potential mechanism has not been adequately described with regards to affecting the susceptibility to colorectal cancer. The present study aimed to evaluate the effects of the *BCRP* variants on the susceptibility to colorectal cancer and to predict the individual responses to xenobiotics transferred by *BCRP*. *BCRP* 421C>A was significantly associated with the colorectal cancer risk (odds ratio, 16.12; P=0.005). These findings are the first results of *BCRP* allele distributions in the Turkish population and provide an understanding of the correlation between therapeutic approaches and etiology of colorectal cancer.

Introduction

Colorectal cancer, one of the most challenging health issues today, is a multi-factorial disease that results from complex interactions between environmental and genetic factors (1-3). A number of studies have indicated that colorectal cancer is a result of xenobiotic exposure, such as those taken up with diet, cigarette-smoke, drugs and alcohol. The xenobiotics causing colorectal cancer development enter the body as (pro)carcinogens via transporters and are either activated to carcinogens or eliminated by various enzymes (3,4). The genetic background controls these toxicokinetic-associated proteins and enzymes. The genetic variables, ranging from single-nucleotide polymorphism (SNP) to major chromosomal aberration may increase the contribution to the development of cancer (2,5).

The adenosine triphosphate-binding cassette (ABC) transporter superfamily is one of the largest and most broadly-expressed, with the majority of proteins responsible for the active transport of a wide variety of compounds across extra- and intracellular membranes (6-8). The breast cancer resistance protein (*BCRP*) belongs to a subfamily of the ABC transporters and is expressed in the placenta, liver, brain, small intestine and breast in humans (9,10). *BCRP* is predominantly found in the liver canalicular membrane and the apical membrane of the intestinal epithelium. Therefore, *BCRP* transport activity has a critical role in prevention of intestinal absorption and in mediating hepatobiliary excretion of its substrates, including anticancer drugs and carcinogenic xenobiotics (11).

BCRP has undergone systematic screening for SNPs in 90 different ethnic populations. Thus far, >40 non-synonymous and synonymous SNPs have been identified in promoter, and exon and intron sequences (12). Two SNPs, 34G>A and 421C>A, decrease the clearance and/or increase the oral bioavailability of the *BCRP* substrates due to altered transport function and hindered expression of variant *BCRP* proteins in the liver and small intestine. The *BCRP* 34G>A variant (rs2231137), resulting in a Val12Met (V12M) substitution, results in apical plasma membrane dislocalization of *BCRP* and generates a protein with a significantly reduced ability to transport several drugs/xenobiotics. The *BCRP* 421C>A variant (rs2231142), causes a Gln141Lys (Q141K) change, and is associated with low levels of *BCRP* expression (13).

The potential mechanism of the *BCRP* variants affecting susceptibility to colorectal cancer remains to be adequately described (3,14-18). However, based on the current evidence, we hypothesize that two functional and common SNPs of *BCRP*, namely 34G>A and 421C>A, should potentially have an effect on the susceptibility and prognosis of disease. In the present study, the first aim was to determine the significance of *BCRP* in the development of colorectal cancer. The second aim was to predict the individual response of the Turkish population to xenobiotics transferred by *BCRP*, as the *BCRP* alleles, observed in various populations and sub-populations, have not been investigated in the Turkish population.

Correspondence to: Dr Gul Ozhan, Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Istanbul University, Beyazit, Istanbul 34116, Turkey
E-mail: gulozhan@istanbul.edu.tr

Key words: BCRP variants, genetic polymorphisms, colorectal cancer

Materials and methods

Subjects. To examine the association between the *BCRP* gene polymorphisms and the susceptibility to colorectal cancer, a case-control study was conducted in the Turkish population. The study was approved by the Ethics Committee of Istanbul University (2011/87-555; Istanbul, Turkey) and all the participants provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki (1964). Blood samples were collected from 108 patients with colorectal cancer and 157 healthy volunteers that were admitted to the Hospital of Istanbul University and Bagcilar Training and Research Hospital (Istanbul, Turkey) during the same period (between 2011 and 2013). The criteria for diagnosis of colorectal cancer were a clinical history consistent with the disease, positive colonoscopic results and routine laboratory analysis parameters. The histopathological examinations were evaluated according to the established clinical criteria (19). The controls were hospital patients with various diagnoses (eye, pulmonary and cardiovascular diseases, and neurological disorders) who have never had cancer. For all the subjects, gender, age and body mass index (BMI, kg/cm^2) were recorded. There were no significant differences for gender, age and BMI between case (48 females and 61 males; mean age, 43.3±16.0 years; mean BMI, 26.0±4.7 kg/cm^2) and control (87 females and 75 males; mean age, 51.2±17.0 years; mean BMI, 26.7±5.3 kg/cm^2) groups, suggesting that the matching based on these two variables was adequate (P>0.05).

Genotyping of the BCRP variants. Venous blood was drawn from subjects and genomic DNA was extracted from whole blood using standard phenol-chloroform extraction protocols. Genotyping of *BCRP* 34G>A and 421C>A variants was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism methods (Table I). The temperature was controlled by a programmable heat block (Gene Amp PCR System 9700; Applied Biosystems, Carlsbad, CA, USA). Restriction enzymes were obtained from New England Biolabs (Hitchin, UK) and Fermentas (Vilnius, Lithuania). All the other molecular biological chemicals were obtained from Fermentas and Sigma-Aldrich (St. Louis, MO, USA). Genotyping was performed blinded to case-control status. A 10% random sample was genotyped twice for quality assurance, which yielded 100% concordance.

Statistical analysis. The Hardy-Weinberg equilibrium was investigated using the χ^2 test. For the analyses of the genotype frequencies, the wild-type category (chosen either as the most common wild-type frequency or arbitrarily if the two alleles showed similar frequencies) was used as the reference group. The odds ratios (ORs) and 95% confidence intervals (CIs) were estimated based on the comparison of the genotypes between the patients with colorectal cancer and the healthy controls. Statistical analysis was implemented using the Statistical Package for Social Sciences program (version 17.0; SPSS, Inc., Chicago, IL, USA). A two-sided P<0.05 was considered to indicate a statistically significant difference.

Results

BRCP variants. The genotype distributions did not significantly deviate from the Hardy-Weinberg equilibrium in the cases and controls for any of the examined SNPs, *BCRP* 34G>A and 421C>A. Subsequently, the differences between the cases and controls regarding the distribution of genotype was analyzed. The *BCRP* 34A recessive allele frequencies were 0.064 and 0.111 in the controls and cases, respectively. The *BCRP* 421A recessive allele frequencies were 0.139 and 0.013 in the controls and cases, respectively. *BCRP* 421AA genotypes were not observed in the controls and cases (Table II). In all the samples (n=265), the major allelic frequencies for *BCRP* 34G and 421C were observed as 0.922 and 0.940, respectively (Table II).

In the study, it was observed that *BCRP* 421C>A was statistically significantly associated with the colorectal cancer risk. The association was observed with *BCRP* 421C>A (P=0.0005); in particular, patients carrying the *A* allele compared to patients carrying the *C* allele had a significantly higher risk of disease (OR, 16.12; 95% CI, 2.08-125.1) (Table II). To confirm the genotyping results for this variants, which is associated with colorectal cancer, the selected PCR-amplified DNA samples (n=4, for each genotype in the cases and controls) were examined by DNA sequencing and the results were also 100% concordant. By contrast, genotype distribution of *BCRP* 34G>A in the cases and controls did not significantly differ and thus the polymorphism was not associated with the risk of colorectal cancer (OR, 0.52; 95% CI, 0.18-1.46; P=0.204) (Table II).

Discussion

BCRP plays an important role in preventing intestinal absorption, mediating hepatobiliary excretion and controlling the cellular export for its substrates as it actively transports a wide-spectrum of substrates, ranging from chemotherapeutic agents to carcinogenic xenobiotics (20-25). The differential xenobiotic metabolism, which is due to variations in the transporter molecules, may affect the risk of certain types of cancers (26-31). In certain studies, a possible role of transporter deficiencies was indicated in the susceptibility for colon carcinoma (14,18,32). Therefore, 34G>A and 421C>A polymorphisms in the *BCRP* gene were studied as they are the most common between the different ethnic groups (33). 421C>A is associated with the decreased expression of the *BCRP* protein (34) and 34G>A is associated with decreased *BCRP* transporter activity (35).

Dietrich *et al* (3) analyzed *BCRP* expression in 29 adenomas from 21 patients and eight adenomas from four mice. The study reported that *BCRP* was significantly downregulated in human colorectal adenomas (to 28±35% of the adjacent healthy tissue), which may reduce the xenobiotic resistance of cells that are already neoplastic, resulting in acceleration of carcinoma development. Gupta *et al* (16) reported that *BCRP* mRNA was present in normal colorectal tissue, but showed a 6-fold decrease in cancerous tissues. The study suggested that decreased *BCRP* expression may have a role in carcinogenesis by permitting the accumulation of genotoxins and overproduction of nitric oxide, and that downregulation of *BCRP* was likely to be a common

Table I. Details of methodology used in genotyping by polymerase chain reaction analysis.

BCRP SNP	Primer sequence	Annealing temperature, ˚C	Restriction enzyme	Fragment length, bp
421C>A	5'-GTTGTGATGGGCACTCTGATGGT-3' 5'-AACAATGAGAAAAGTGGCTTG-3'	62.4	*Mse*I	289, 191, 98, 64, 34
34G>A	5'-CAGTAATGTCGAAGTTTTTATCGCA-3' 5'-AAATGTTCATAGCCAGTTTCTTGGA-3'	61.2	*Bsr*DI	291, 261, 30

SNP, single nucleotide polymorphism; BCRP, breast cancer resistance protein; bp, base pair.

Table II. Allele frequencies of breast cancer resistance protein (*BCRP*) polymorphisms in the healthy subjects and the patients with colorectal cancer.

Subject (n)	Genotype frequencies, n (%)			Allele frequency		OR (95% CI)	P-value
	BCRP 421C>A						
	CC	CA	AA	C	A		
Cases (108)	0 (0)	30 (27.8)	78 (72.2)	0.139	0.861	C vs. A	0.0005
Controls (157)	0 (0)	2 (1.3)	155 (98.7)	0.013	0.987	16.12 (2.08-125.1)	
	BCRP 34G>A						
	GG	GA	AA	G	A		
Cases (108)	85 (78.7)	22 (20.4)	1 (0.9)	0.889	0.111	G vs. A	0.204
Controls (157)	138 (87.9)	18 (11.5)	1 (0.6)	0.936	0.064	0.52 (0.18-1.46)	

OR, odds ratio; CI, confidence interval.

occurrence in several tissues. Liu *et al* (36) believed that *BCRP* expression may be different in the various stages of carcinogenesis. In the early stage of carcinogenesis, *BCRP* may be downregulated to permit the accumulation of genotoxins and nitric oxide overproduction. However, in the more advanced stages of carcinogenesis, it may be upregulated to protect the cancerous cells by expelling the chemotherapeutic drugs. The study concluded that *BCRP* was important in the progression and metastasis of colorectal cancer and that it may be a novel target for cancer therapy. Ebert *et al* (15) observed that *BCRP* was involved in the transport of the metabolically formed sulphate and glucuronide conjugates of the carcinogen benzo[a] pyrene and related food-associated polycyclic aromatic hydrocarbon (PAH) in the Caco-2 human intestinal cell line. The study also reported that *BCRP* gene regulation was most likely mediated via an Ah receptor-dependent pathway.

Hu *et al* (37) investigated the association between the risk and survival of diffuse large B-cell lymphoma (DLBCL) and the two *BCRP* variants, 421C>A and 34G>A. An increased risk of DLBCL was associated with the 421A allele (P=0.042) and a worse survival was associated with the 34AA genotypes (hazard ratio, 3.69) in 156 DLBCL patients and 376 control subjects. In another study, a Japanese group found that the *BCRP* 421CC homozygotes were at a higher risk to develop non-papillary renal cell carcinoma (30). In the present study, *BCRP* 421C>A was significantly associated with the colorectal cancer risk (OR=16.12, 95% CI: 2.08-125.1), whereas distribution of the *BCRP* 34G>A genotypes did not significantly differ in the case and control groups (P=0.204). In contrast to the present study results, Andersen *et al* (1) found no association between the *BCRP* genotypes and risk of colorectal cancer, and no interaction between *BCRP* 421C>A and meat, smoking or nonsteroidal anti-inflammatory drugs. In the study by Zamber *et al* (33), there was no significant correlation between the 421C>A variant and *BCRP* expression in human intestinal samples.

The previous studies have shown significant differences in the genotype frequencies of 34G>A and 421C>A among different ethnic populations. For 34G>A, the Han Chinese population appears to exhibit the highest frequency (34%), whereas the variant allele is extremely rare in the sub-Sahara African populations (<1%) and occurs at a relatively low frequency in African-American and Caucasian populations in the United States (5 and 12%, respectively) (38). The allelic frequencies in Mexican-Indian, Mexican and Hispanic populations were 90, 10 and 40%, respectively (33). The 421C>A allele appears to be extremely common in Asian populations, with allelic frequencies reported between 27 and 34% (39-41). The 421C>A allele is the most prevalent allele in Japanese and Chinese populations with an allelic frequency of 35% (40,42).

In contrast, the 421C>A allele is rare in sub-Sahara African and African-American populations, with a frequency of 5% (39,41). The frequency in Caucasian populations is ~10% (35,41,43,44). For the two polymorphisms investigated in the present study, there was no prior information regarding their distribution in the Turkish population. The study showed that the frequencies of the Turkish population who exhibited the 421A and 34A alleles were 6 and 8.3%, respectively. Regarding these results, approximately one in ten Turkish subjects may express low amounts of *BCRP* and Turkish people may be vulnerable when assessing the sensitivity of the population to therapeutic drugs and environmental toxicants.

In conclusion, it is widely believed that the *BCRP* transporters play a crucial role in detoxification and protection against xenobiotic substances. There may be cases in which the *BCRP* gene variants may be more or less important contributors regarding the sensitivity to cancer-inducing or -promoting compounds, by interacting differently to the environmental factors. These findings assure further investigations with regards to the association of the *BCRP* SNPs with with susceptibility and the clinical outcome of cancer. To the best of our knowledge, this is the first study to report the association between the two polymorphisms and colorectal cancer. Therefore, the results of the present study suggest that the findings contribute to the literature, even when the small number of subjects examined with the association may have limited the power to detect the effects of the gene on the colorectal cancer risk.

Acknowledgements

The authors wish to thank all the subjects that volunteered to participate. The study was supported by the Research Fund of Istanbul University (grant no. 24985/42545).

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