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Contribution of Genetic Variants of ABC Transporters (*ABCC1* and *ABCG2*) genes with the Pathogenesis of Colorectal Cancer

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Colorectal cancer (CRC) is one of the major cancers that is characterized with high percentage of morbidity worldwide due to the advanced metastatic cancer that developed via acquired drug resistance mechanisms. Therefore, there is an urgent need to identify genetic variants in major genes that could contribute to the poor overall survival rate and drug-resistance. ATP-binding cassette (ABC) transporters are among the most studied genes that are related to the development of many cancers including CRC. In this study, three variants namely (G2168A and G3173A) in *ABCC1* and (C421A) in *ABCG2* were examined to evaluate their contribution to CRC in Saudi Arabia. DNA was extracted from the whole blood of 62 CRC patients and 100 controls. PCR-RFLP technique was used to identify the different genotypes among Saudi population. All statistical data were obtained by chi-square test and P values<0.05 were considered statistically significant. Interestingly, neither of the tested variants showed heterozygous nor homozygous distribution among the 162 samples. Therefore, those variants are rare in Saudi population and are not suspected to be involved in CRC pathogenesis. In conclusion, those variants cannot be used as diagnostic or prognostic markers for CRC in Saudi Arabia. However, more experiments need to be performed to confirm our findings.

Keywords: Colorectal cancer (CRC), single nucleotide polymorphisms (SNPs), development risk, Saudi Arabia.

Despite extensive and effective measures of preventive screening, as well as significant advances in treatment options, colorectal cancer (CRC) remains one of the most widespread and fatal cancers in both men and women worldwide. Based on the latest statistical analysis report, CRC considered the third most common malignancy and the fourth leading cause of cancer-related deaths worldwide, as 1,400,000 new cases are diagnosed yearly and about 700,000 deaths occur[1]. In Saudi Arabia, according to the analysis report from National cancer registry (NCR), it ranks the first

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and the third most common cancers among males and females, respectively[2]. Because CRC patients often have symptoms in the early stage of the disease, the diagnosis at this stage represents an important clinical challenge as it might increase the response of the patients to the currently used treatments and therefore, their survival rates. The identification of the role of genetic variants in major genes that usually expressed highly in cancer patients are among the most recently diagnostic methods.

ATP-binding cassette (ABC) transporters are major proteins that play several important physiological functions. They act as exporters and importers for several natural and chemical molecules. There are 49 proteins identified in human, 14 of which are associated with various diseases. They are classified into seven subgroups named ABCA to ABCG depending on amino acid sequences[3,4,5]. In cancer research, three major ABC transporters (ABCB1, ABCC1, and ABCG2) are widely studied for their contribution to the tumorigenesis of many cancers.

Multidrug resistance-associated protein 1 (MRP1/ABCC1) is the first identified member of ABCC subfamily, which belongs to ATP-binding cassette (ABC) transporter superfamily[6,7]. Subsequent studies revealed the mechanism of MRP1/ABCC1 as an exporter of drugs and metabolites in many physiological, pathological and pharmacological processes[8]. *MRP1/ABCC1* gene is located on chromosome 16p13.1 and spans 531 amino acid residues with an apparent molecular weight of 180-190kD[6]. With the advance in sequencing technology, many *ABCC1* single nucleotide polymorphisms (SNPs) and common haplotypes were identified in different human populations. On the other hand, breast cancer resistant protein (BCRP/ABCG2) belongs to the G-subfamily of the ABC transporters. This transporter is able to transport different compounds across cellular membranes, therefore, it plays an important protection role against many toxins. It is located on chromosome 4q22, and encodes a half-transporter that contains 655 amino acid polypeptide. Besides the expression of ABCG2 in human cell lines from breast, colon, ovary, and gastric cancer, the distribution of ABCG2 has been located in human colon, small intestine, heart and brain[9]. Therefore, this research was interested in finding the role of genetic variants in *ABCC1* and *ABCG2* genes, particularly, SNPs G2168A and G3173A in *ABCC1* gene as well as SNP C421A in *ABCG2* gene, on the pathogenesis of CRC in Saudi patients as less is known about their correlations with CRC.

MATERIALS AND METHODS

Materials

QIAamp DNA Blood Mini Kit (catalog number: 51106, QIAGEN Inc., USA). QIAGEN HotStarTaq Master Mix Kit (catalog number: 203601, Valencia, CA, USA). Restriction enzyme Hin4I (catalog number: ER1601, Fermentas, USA). Restriction enzyme BstEII (catalog number: R0162S, NEB, USA). Restriction enzyme HpyCH4III (catalog number: R0618S, NEB, USA). Thermo Scientific GeneRuler 100 bp DNA ladder (catalog number: SM0243, ThermoFisher Scientific, USA).

Subjects and samples

In this study, 62 CRC patients and 100 healthy controls were involved. The purpose of the research was explained and a written consent of the participants as well as their answers on a questionnaire were obtained to be involved in the study. The General Directorate of Health Affairs in Jeddah and the unit of biomedical ethics at KAU Faculty of Medicine approved this study. Whole blood samples were drawn into (EDTA) lavender top vacutainers. All samples were collected from CRC patients and controls who were routinely visiting the oncology centers and blood bank units at King Abdullah Medical city, King Abdulaziz University Hospital (KAUH) and King Fahad General Hospital in Jeddah, Saudi Arabia.

DNA extraction and genotyping of SNPs

Genomic deoxyribonucleic acid (gDNA) was extracted from peripheral blood leukocytes in whole blood samples using QIAamp DNA Mini Kit following the manufacturer's instructions. The DNA concentration was determined by reading the absorbance at a wavelength of 260 nm using the Thermo Scientific NanoDrop 2000 spectrophotometer. Purity was determined by calculating the ratio of absorbance at 260/280 nm and 260/230 nm. Then, PCR was used to amplify the different regions that contains the desired SNPs in both genes. For a 25µl PCR reaction, 1µl

genomic DNA (100 ng/µl), 12.5µl HotStarTaq Master Mix, 9.5µl RNase free water, and 1µl of each forward and reverse primer (Table 1) were used. The PCR thermocycler program was as follow: initial denaturation was done at 95ºC for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 64, 60, and 62°C for 1 min for SNPs G2168A, G3173A, and C421A, respectively, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. PCR products were run on 1.5% agarose gel to determine the amplification. Then, all amplified PCR products were treated with the different endonucleases following the manufacturer's instructions to reveal the different genotypes with restriction fragment length polymorphism (RFLP) analysis (Table 2).

Statistical analysis

A χ^2 – square test was used to calculate the allele frequency and the genotype distribution of each variant with that expected for a population in Hardy- Weinberg equilibrium. *P* values of <0.05 were considered statistically significant.

RESULTS

Genotypes distribution of *ABCC1* and *ABCG2* SNPs

The genotype and allele frequencies of the three SNPs in *ABCC1* and *ABCG2* genes were examined by RFLP for all subjects (n=162). Interestingly, the results showed 100% normal genotype in both patients and controls. The two other genotypes; heterozygous and homozygous were not detected in all patients and controls which indicates that our population are under Hardy-Weinberg equilibrium assumption (*P*>0.05) and the rarity of those SNPs in Saudi population.

Table 1. Primers sequence and PCR product sizes used for detection of *ABCC1* and *ABCG2* SNPs

SNP ID	Primers	PCR products sizes
ABCC1 G2168A	Forward Primer 5'-TATCTTTCGGTCCACCGTAACTG-3' Reverse Primer 5'-GGGGCCAACATCCAACCTAT-3'	587 bp
ABCC1 G3173A	Forward Primer 5'-ACCTCGGCCTCCCAAAGTGCT-3' Reverse Primer 5'-CAAGGGGCGGGATGATGATGG-3'	466 bp
ABCG2 C421A	Forward Primer 5'-TTGGGTGTACAGATAGGGGGTGAAA-3' Reverse Primer 5'-ATAAGGAAAGGCCAGGTGATGAACA-3'	632 bp

Table 2. Genotypes length after treatment with endonucleases

SNP ID	Restriction enzyme	Length after RFLP
ABCC1 G2168A	Hin4I	Major allele: 320, 128, 96, and 45 bp Minor allele: 448, 96, and 45 bp
ABCC1 G3173A	BstEII	Major allele: 272 and 194 bp Minor allele: 466 bp
ABCG2 C421A	HpyCH4III	Major allele: 376, 88, 87, 66, and 17 bp Minor allele: 393, 88, 87, and 66 bp

*RFLP; restriction fragments length polymorphism

DISCUSSION

Colorectal cancers (CRC) is one of the most commonly Diagnosed cancers worldwide. Chemotherapy is the one of most common treatment regimen for CRC, but tumor resistance causes its failure[10,11]. The more common causes of resistance for chemotherapies is the increased expression of ABC transporters such as ABCC1 and ABCG2. In this study, the genotype distribution of three major SNPs in *ABCC1* (G2168A and G3173A) and *ABCG2* (C421A) were examined to evaluate their contribution to CRC risk in Saudi Arabia. Interestingly, all tested SNPs showed non-significant correlation to CRC risk as they were all normally distributed as shown by chi-square analysis test. Single nucleotide polymorphisms in the *Athe ABCC1 and ABCG2 genes* were found to change the expression and activity of the corresponding gene and its product. Therefore, they may increase the risk and affect the response of the cancer cells to the therapy and result in increased susceptibility to different types of cancers[12,13]. The exact effect of these variants is inconsistent which make it difficult to conclude the exact role of these variants in cancer. The SNPs G2168A and G3173A in *ABCC1* gene have been studied in many diseases and to lesser extent in cancer. In most of these studies, they were interested in determining their role in drug resistance more than their ability to increase the risk of the disease. Therefore, it was difficult to compare our results with the published results. Regarding SNP C421A, less publications were found that conclude the correlation between this SNP with the risk of many cancers. In contrast to our finding, Wu and his team found that carriers with *ABCG2* G34A A allele and C421A A allele and haplotype G34A A-C421A C or G34A G-C421A A were significantly associated with the increased breast carcinoma risk[14]. The variant 421C>A or 34G>A was associated with an increased incidence risk of diffuse large B- cell lymphoma (DLBCL)[15]. However, the 421C>A allele was reported to decrease risk for renal cell carcinoma[14] and cannot be used as a marker for the gefitinib-toxicity in non-small cell lung cancer (NSCLC)[16]. Moreover, Li *et al.,* (2017) found that Chinese breast cancer patients with normal genotype for SNP 421 CC in *ABCG2* gene had increase progression-free survival (PFS)[17].

In conclusion, the current study to the best of our knowledge, is the first study that correlates variants in *ABCC1* and *ABCG2* genes with the risk of CRC in Saudi Arabia. The three studied variants showed normal genotypes distribution among Saudi population and therefore did not show a high risk for CRC development or pathogenesis. However, this study needs to be done on a larger population size and to be performed on tissue samples to confirm the findings and to elucidate the possible mechanisms behind the protective role.

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