# Association between *MDR1* polymorphisms and XELIRI and XELOX chemoresistance in Saudi patients with colorectal cancer

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**Abstract.** Multidrug resistance member 1 (MDR1) is located on chromosome 7 and encodes P-glycoprotein, which is universally accepted as a drug resistance biomarker. *MDR1* polymorphisms can alter protein expression or function, which has been previously reported to associate with various types of malignancies, such as colorectal cancer (CRC). Therefore, the present study aimed to determine the effects of *MDR1* polymorphisms on drug responses of Saudi patients with CRC. DNA samples were obtained from 62 patients with CRC and 100 healthy controls. Genotypes and allele frequencies of *MDR1* single nucleotide polymorphisms (SNPs) G2677T and T1236C were determined using the PCR-restriction fragment length polymorphism procedure. The results showed no significant differences in the genotype distribution and allele frequency of T1236C between patients with CRC and controls. However, G2677T was found to serve a highly significant role in protecting against the progression of CRC. In addition, none of the genotypes in SNPs T1236C and G2677T was found to affect chemoresistance to XELIRI and XELOX. In conclusion, although T1236C in the *MDR1* gene is not associated with CRC risk, G2677T protects against the development of CRC. Neither of the *MDR1* SNPs tested were associated with the risk of chemoresistance. Therefore, these two SNPs cannot be used as molecular markers for predicting drug response in patients with CRC.

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## Introduction

Colorectal cancer (CRC) is ranked as the 3rd most common prevalent malignancy and the 4th most common cause of cancer-associated mortality worldwide in 2016 (1,2). According to the latest annual cancer incidence report from the Saudi Cancer Registry in 2015, CRC is the most common cancer among men and the 3rd most common cancer in women (3). Although CRC treatment strategies have evolved in recent years, they remain ineffective in certain patients for various reasons, including changes in the absorption capacity, metabolism or drug uptake of target cells and development of drug resistance to multiple anticancer agents (4). Cancer multidrug resistance (MDR) occurs when cancer cells are treated with primary chemotherapy or in recurrence following primary chemotherapy (4). The occurrence of cancer MDR has numerous underlying mechanisms, including increased efflux of drugs through cellular transporters (5).

The ATP-binding cassette (ABC) transporter family serves an important role in cancer MDR (4,5). The family of human ABC transporters consists of 49 members (5), which are divided into seven subfamilies, A to G (4). The ABCB subfamily is a subclass of ABC transporters that has 11 members, one of which is ABCB1, also known as multidrug resistance member 1 (MDR1) (6). MDR1 was the first human ABC transporter to be cloned and characterized with regards to its ability to confer MDR phenotypes on cancer cells (6). The *MDR1* gene is located on chromosome 7 and encodes P-glycoprotein (Pgp), which is universally accepted

as a drug resistance biomarker (5,7). The *MDR1* gene is highly expressed in numerous regions, including the proximal and distal human intestines, which causes the excretion of certain carcinogens such as taxanes, anthracyclins, *vinca* alkaloids, and epipodophyllotoxins from the gut into the intestinal lumen (5). Pgp serves an important role in the detoxification of healthy tissues, as it transports substrates and protects tissues from physiologically active substances, cytotoxic agents and xenobiotics (5,8).

The expression of the *MDR1* gene and the activity of its protein product can differ among individuals due to genetic single nucleotide polymorphisms (SNPs) (7-11). Since gene polymorphisms have the ability to influence clinical response to chemotherapy, they can also affect the absorption, distribution, metabolism and excretion of drugs (9-11). The *MDR1* gene has numerous reported mutations, where 40 of its SNPs have been previously described for exons, introns and promoters (7-11). Numerous studies have demonstrated that several *MDR1* variants are associated with the increased risk of CRC, including the G2677T and T1236C polymorphisms (8,9,11-15). The G2677T SNP of the *MDR1*, located in exon 21, converts alanine to serine or threonine, which affects the pump function of Pgp (8). By contrast, the T1236C SNP is one of the most common polymorphisms in the *MDR1* gene and is located in exon 12, which is a silent mutation, similar to that of the *MDR1* SNP C3435T mutation (8).

A number of studies have reported that SNPs in the *MDR1* gene contribute to the risk of developing CRC in several ethnic groups, either by changing the structure and function of the pump or by affecting the response of cancer cells to the drugs currently in use (7-15). Chemotherapeutic drugs for CRC have evolved in recent years which exhibit promising signs in extending the survival rates for patients with CRC. XELIRI™ and XELOX™ are widely recommended for patients with CRC (16,17). XELIRI is composed of two chemotherapeutic drugs, Xeloda and irinotecan, which is commonly used to treat various types of malignancies such as metastatic CRC and advanced stomach cancer (16,18). It is frequently administered to patients in cycles, with each cycle lasting 2-3 weeks, depending on the extent of the disease. Patients usually take Xeloda orally in tablet form and irinotecan by intravenous injection (16). XELOX is a chemotherapeutic agent that is comprised of Xeloda and oxaliplatin. It is widely used for the treatment of bowel cancers, including CRC (17). As with the XELIRI treatment regimen, patients with CRC usually take Xeloda orally in tablet form and oxaliplatin intravenously (17). Both drugs kill cancer cells by exerting toxic effects that cause DNA damage, though their mechanisms of action differ (16,17).

The present study aimed to determine the genotypic distribution and allele frequency of two major *MDR1* SNPs, T1236C and G2677T, in Saudi patients with CRC. The genetic results were then compared with responses to major chemotherapeutic drugs, XELIRI and XELOX, which were used to treat patients with metastatic CRC.

## Materials and methods

*Subjects and samples.* A total of 162 volunteers (age mean 54.10±0.96 years) participated in the present study from January 2015 to December 2015. The participants were subsequently divided into two groups: 62 patients with CRC (n=48 males and n=14 females) and 100 healthy controls (n=70 males and n=30 females). The CRC subjects were included if they were Saudis with a confirmed histopathological diagnosis of CRC at any TNM stage. Any non-Saudis metastatic CRC patient (i.e. having CRC as a secondary tumor) was excluded. On the other hand, healthy control subjects included Saudis, free of any metabolic or chronic diseases or inflammation, without any family history of CRC or any other tumor. Blood samples from patients with CRC were collected from King Abdulaziz University Hospital (Jeddah, Saudi Arabia), whilst samples from healthy controls were collected from the blood bank unit of King Fahad General Hospital (Jeddah, Saudi Arabia). The clinicopathological features of the patients with CRC are listed in Table I. In accordance with the TNM staging system (19), the patients were divided clinically into stages I (n=7), II (n=6), III (n=17) and IV (n=32). Patients at stage I did not receive chemotherapy treatment whereas those at stages II, III and IV received chemotherapy. Patients at non-metastatic stages II, III and IV received either XELIRI treatment [irinotecan hydrochloride (Merck & Co., Inc.) intravenously (250 mg/m$^2$) and Xeloda tablets orally (1,000 mg/m$^2$) twice per day (Roche Diagnostics)] for 2-3 weeks, or XELOX treatment [oxaliplatin (Merck & Co., Inc.) intravenously (130 mg/m$^2$) and Xeloda tablets orally (1,000 mg/m$^2$) twice per day] for 3 weeks. Patients at metastatic stages III and IV received a combination of the same doses of XELIRI and XELOX with bevacizumab (Roche Diagnostics) intravenously (5 mg/kg) every 2 weeks. Blood levels of carcinoembryonic antigen (CEA), a CRC tumor marker, were measured twice at Biochemistry lab in King Abdulaziz University Hospital using ARCHITECT CEA chemiluminescent assay (Abbott Pharmaceutical Co. Ltd.) and follow-up CT-scan imaging was performed following the completion of the chemotherapy cycles to assess the response of cancer cells to treatment with either XELIRI or XELOX (20). Based on the CEA level, the patients were divided into either drug-resistance (>3 ng/ml) or drug-response (<3 ng/ml).

The aim of the present study was explained to the participants, following which written informed consent and questionnaires including anthropometric measurements, smoking status, nutritional status, and family history of cancer and medications were obtained from all participants. The present study was approved by the General Directorate of Health Affairs in Jeddah, Saudi Arabia (approval no. A00221).

Whole blood samples (2 ml) were collected from each participant and subjected to DNA extraction using the QIAamp DNA mini kit (Qiagen GmbH), according to the manufacturer's protocol. The range of DNA concentrations were 3-12 $\mu$g with a purity of 1.7-1.9. These measurements were determined by calculating the ratio of absorbance at 260/280 nm using a DeNovix DS-11 spectrophotometer.

*PCR-restriction fragment length polymorphism (RFLP).* PCR was performed in a reaction containing 1 $\mu$l (100 ng/$\mu$l) DNA mixed with 12.5 $\mu$l USB® HotStart-IT® FideliTaq™ PCR Master Mix (2X; Affymetrix; Thermo Fisher Scientific, Inc.), 9.5 $\mu$l RNase free water and 1 $\mu$l (100 nmol) forward and reverse primers. The primer pairs used for PCR amplification

Table I. Clinicopathological characteristics of 62 patients with colorectal cancer.

| Clinicopathological characteristic | N (%)[a] |
|---|---|
| Age | |
| 30-59 years | 41 (66.13) |
| 60-89 years | 21 (33.87) |
| Site of cancer | |
| Colon | 54 (87.10) |
| Rectum | 8 (12.90) |
| Sex | |
| Male | 48 (77.42) |
| Female | 14 (22.58) |
| TNM stage | |
| I | 7 (11.29) |
| II | 6 (9.68) |
| III | 17 (27.42) |
| IV | 32 (51.61) |
| Treatment per TNM stage | |
| I | No chemotherapy; 6 (9.68) |
| Non-metastatic II, III and IV | XELIRI or XELOX; 45 (72.58) |
| Metastatic III and IV | XELIRI, XELOX and bevacizumab; 11 (17.74) |
| CEA per TNM stage (ng/ml) | |
| I | 2.86±0.48 |
| II | 5.89±4.26 |
| III | 8.44±3.58 |
| IV | 12.34±2.72 |

[a]Data are presented as n (%) for age, site of cancer, sex and TNM stage, but as the mean ± SEM for CEA. TNM, cancer staging system; CEA, carcinoembryonic antigen.

were as follows: SNP T1236C in exon 12 forward, 5'-TTTTTC TCACGGTCCTGGTAG-3' and reverse, 5'-CATCCCCTC TGTGGGGTCATA-3' and SNP G2677T in exon 21 forward, 5'-TACCCATCATTGCAATAGCAG-3' and reverse, 5'-TTT AGTTTGACTCACCTTTCTAG-3). The thermocycling conditions were as follows: Initial denaturation at 94˚C for 5 min, followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 60˚C (for SNP T1236C) or 55˚C (for SNP G2677T) for 1 min, extension at 72˚C for 2 min and final extension at 72˚C for 5 min. The amplified PCR products were 147 and 107 bp for SNPs T1236C and G2677T, respectively (21).

The PCR products were digested with endonuclease enzymes *Hae*III and *Xba*I (New England BioLabs, Inc.) at 37˚C for 1 h, followed by an additional inactivation step at 80˚C and 65˚C for 20 min to determine the different genotypes of SNPs T1236C and G2677T, respectively, according to the manufacturer's protocol. RFLPs for SNP T1236C were detected using 1.5% agarose gel stained with ethidium bromide as follows: i) Wild-type (TT), 2 bands, 68 and 79 bp; ii) heterozygous (TC), 4 bands, 33, 35, 68 and 79 bp; and iii) homozygous (CC), 3 bands, 33, 35 and 79 bp. RFLPs for SNP G2677T were detected as follows: i) Wild-type (GG), 1 band, 107 bp; ii) heterozygous (GT), 3 bands, 24, 83 and 107 bp; and iii) homozygous (TT), 2 bands, 24 and 83 bp (21).

*Statistical analysis*. All statistical comparisons were performed using GraphPad Prism software (version 5.0; GraphPad Software, Inc.). All PCR amplification experiments and determination of genotypes by RFLP were performed once, unless, amplification or restricted bands were unclear. One-way ANOVA followed by Bonferroni's multiple comparison post hoc test was used to compare the parametric variables among >2 groups. Mann-Whitney U and unpaired t-test were used to compare the parametric values between two groups only. $\chi^2$ test and Fisher's exact probability test (two-tailed P-values) were applied to determine the genotype distribution and allele frequency of the SNPs as well as odds ratio (OR) and its 95% confidence interval (95% CI) according to Hardy-Weinberg equilibrium equations ($p^2+2pq+q^2=1$ and $p+q=1$ for genotype or allele frequencies, respectively) (22) where p is the dominant allele and q is the recessive allele. All data in tables (demographic data and CEA levels for CRC patients in the same clinical stage) are presented as the mean ± SEM or as n (%). P<0.05 was considered to indicate a statistically significant difference.

## Results

*Demographic distribution of the participants*. A total of 62 patients with CRC [male, n=48 (77.42%); female, n=14 (22.58%)] participated in the present study. The healthy controls (n=100) were divided into males (n=70; 70%) and females (n=30; 30%). Mann-Whitney U test (Table II) was used to compare the physical parameters obtained from patients with CRC and healthy controls. The results demonstrated a significant difference in weight, which affected the body mass index. This was due to loss of appetite in patients receiving chemotherapy.

*Genotypic frequencies of MDR1 SNPs T1236C and G2677T in patients with CRC and healthy controls*. For SNP T1236C, the genotypic frequencies of the patients with CRC were 0% (n=0) for TT, 66.13% (n=41) for TC and 33.87% (n=21) for CC. The frequencies of the T and C alleles were found to be 32.26 and 67.74%, respectively. The genotype distribution for patients with CRC was not within the Hardy-Weinberg equilibrium [$\chi^2$=15.12; degrees of freedom (DF)=1; P<0.05]. The results for the healthy controls demonstrated genotypic frequencies of 0% (n=0) for TT, 46% (n=46) for TC and 54% (n=54) for CC. The frequencies of the T and C alleles were 23 and 77%, respectively. The genotype distribution for the controls was not within the Hardy-Weinberg equilibrium ($\chi^2$=8.92; DF=1; P<0.05). Comparing the genotype frequencies was challenging, since no participants carried the TT genotype. As a result, it was challenging to conclude the association between this SNP and the risk of developing CRC in the participants (Table III).

For SNP G2677T, the genotypic frequencies of the patients were 69.4% (n=43) for GG, 6.5% (n=4) for GT and 24.1% (n=15) for TT. The frequencies of the G and T alleles

Table II. Demographic analysis of all the participants in the current study.

| Physical parameter | Patients with CRC (n=62) | Healthy controls (n=100) | P-value |
|---|---|---|---|
| Age (years) | 55.89±1.60 | 53.00±1.19 | 0.1367 |
| Weight (kg) | 73.37±2.01 | 84.41±1.78 | <0.0001 |
| Height (cm) | 165.50±1.19 | 165.50±0.96 | 0.5414 |
| BMI (kg/m$^2$) | 26.80±0.72 | 30.91±0.60 | 0.0001 |
| Waist (cm) | 100.10±2.54 | 102.80±2.22 | 0.5671 |
| Hip (cm) | 109.80±2.43 | 108.10±1.84 | 0.9546 |
| Waist-to-hip ratio | 0.92±0.02 | 0.96±0.01 | 0.3566 |

Data are presented as mean ± SEM. CRC, colorectal cancer; BMI, body mass index.

Table III. Genotype distribution and allele frequency analysis of the single nucleotide polymorphisms of multidrug resistance member 1.

A, T1236C

| Genotype and alleles | Patients with CRC [n=62; n, (%)] | Healthy controls [n=100; n, (%)] | Fisher's exact P-value | OR (95% CI) |
|---|---|---|---|---|
| Wild-type (TT) | 0 (0) | 0 (0) | | 1.000 (Reference) |
| Heterozygous (TC) | 41 (66.13) | 46 (46) | 1 | N/A |
| Homozygous (CC) | 21 (33.87) | 54 (54) | 1 | N/A |
| Combined (TC+CC) | 62 (100) | 100 (100) | 1 | N/A |
| Dominant (T) | 20 (32.26) | 23 (23) | | 1.000 (Reference) |
| Recessive (C) | 42 (67.74) | 77 (77) | 0.1600 | 0.610 (0.330-1.130) |

B, G2677T

| Genotype and alleles | Patients with CRC [n=62; n, (%)] | Healthy controls [n=100; n, (%)] | Fisher's exact P-value | OR (95% CI) |
|---|---|---|---|---|
| Wild-type (GG) | 43 (69.4) | 1 (1) | | 1.000 (Reference) |
| Heterozygous (GT) | 4 (6.5) | 24 (24) | <0.0001 | 0.004 (0.0004-0.040) |
| Homozygous (TT) | 15 (24.1) | 75 (75) | <0.0001 | 0.005 (0.0006-0.040) |
| Combined (GT+TT) | 19 (30) | 99 (99) | <0.0001 | 0.005 (0.0006-0.030) |
| Dominant (G) | 45 (72.58) | 13 (13) | | 1.00 (Reference) |
| Recessive (T) | 17 (27.42) | 87 (87) | 1.6800 | 0.050 (0.020-0.100) |

CRC, colorectal cancer; OR, odds ratio; CI, confidence interval; N/A, not applicable.

were 72.58 and 27.42%, respectively. The genotype distribution in patients with CRC was not within the Hardy-Weinberg equilibrium ($\chi^2$=42.39; DF=1; P<0.05). For the healthy controls, the genotypic frequency results showed 1% (n=1) for GG, 24% (n=24) for GT and 75% (n=75) for TT. The frequencies of the G and T alleles were 13 and 87%, respectively (Fig. S1). The genotype distribution for the controls was within the Hardy-Weinberg equilibrium ($\chi^2$=0.59; DF=1; P>0.05). Based on the calculated OR, the results demonstrated that SNP G2677T had a significant role in protecting against the development of CRC, particularly when comparing the GT and TT frequencies with participants carrying the GG genotype in both groups (Table III).

*Relationship between genetic variations in the MDR1 gene and drug responses in patients with CRC*. To assess the association between the genetic variants of the patients with their drug responses, the genotype distribution and allele frequency of the two *MDR1* SNPs were compared against the levels of CEA, which is a major CRC tumor marker used to determine patient response to treatment (20). CEA levels and follow-up CT scan imaging demonstrated that among the 62 patients, 36 were drug resistant (CEA level >3 ng/ml). The drug-sensitive and -resistant patients with each genotype were compared using an unpaired t-test and a final comparison was performed for the six genotype groups using a one-way ANOVA test

Table IV. Association between CEA level and genotypes of SNPs T1236C and SNP G2677T in *MDR1* gene with drug response.

A, SNP T1236C

| Genotype in *MDR1* gene | CEA level (ng/ml) | Unpaired t-test P-value[a] | One-way ANOVA P-value[b] |
|---|---|---|---|
| Wild-type (TT) drug resistant (n=0) | 0 | N/A | |
| Wild-type (TT) drug sensitive (n=0) | 0 | | |
| Heterozygous (TC) drug resistant (n=21) | 576.80±385.90 | 0.15 | 0.27 |
| Heterozygous (TC) drug sensitive (n=20) | 2.01±0.15 | | |
| Homozygous (CC) drug resistant (n=15) | 51.73±29.65 | 0.30 | |
| Homozygous (CC) drug sensitive (n=6) | 1.27±0.16 | | |

B, SNP G2677T

| Genotype in *MDR1* gene | CEA level (ng/ml) | Unpaired t-test P-value[a] | One-way ANOVA P-value[b] |
|---|---|---|---|
| Wild-type (GG) drug resistant (n=25) | 498.50±325.30 | 0.20 | |
| Wild-type (GG) drug sensitive (n=18) | 1.91±0.15 | | |
| Heterozygous (GT) drug resistant (n=3) | 25.64±8.22 | N/A | N/A |
| Heterozygous (GT) drug sensitive (n=1) | 2.45±0.00 | | |
| Homozygous (TT) drug resistant (n=8) | 43.85±30.58 | 0.22 | |
| Homozygous (TT) drug sensitive (n=7) | 1.57±0.30 | | |

[a]Unpaired t-test was used to calculate the significant differences between drug resistant and drug sensitive CRC patients carrying the same genotype (2 groups) in each SNP. [b]One-way ANOVA test was used to compare the differences between drug resistant and drug sensitive CRC patients of all genotypes in each SNP (6 groups in total). Data are presented as mean ± SEM. CEA, carcinoembryonic antigen; SNPs, single nucleotide polymorphisms; MDR1, multidrug resistance member 1; N/A, not applicable.

with Bonferroni's multiple comparison post-hoc test. Table IV presented the mean ± SEM of CEA levels for each genotype and its association with drug resistance for SNPs T1236C and G2677T in patients receiving XELIRI and XELOX. The results revealed that none of the genotypes of either of the two SNPs in the *MDR1* gene increased the risk of developing chemoresistance to either XELIRI or XELOX, as shown by the non-significant P-values.

## Discussion

There are numerous advantages in using SNP analyses and arrays to detect novel single nucleotide variants (SNVs), including trait associations with diseases in discovering novel biological mechanisms and identifying novel ethnic variations that may affect patient response to therapy (23). However, various limitations can be present in these analyses, including the continuous need for the application of statistical corrections to adopt a high level of significance to account for multiple tests. Additionally, these analyses only reported a fraction of the missing heritability and do not necessarily pinpoint the causal variants and genes (23). Nevertheless, these analyses have been successful in identifying novel biomarkers for many diseases including cancers, type II diabetes mellitus and anorexia nervosa (23). In the present study, the SNP T1236C in the *MDR1* gene and its potential contribution to the risk of developing CRC and drug resistance was investigated. Although the genotype distribution, allele frequency, OR and P-values were calculated using $\chi^2$ test, genotype frequencies could not be compared as none of the participants in either the patient or control groups carried the TT genotype. Therefore, determining the association between this SNP and the risk of developing CRC was challenging. However, several previous studies have assessed the association between the T1236C polymorphism and CRC risk with contradictory results (8,24).

The other SNP that was investigated in the present study was G2677T. The results demonstrated that this SNP served a significant role in protecting against the development of CRC, particularly when comparing the patients with CRC and the control group with regards to the GT and TT genotypes (Table III). This demonstrated that the participants carrying the GT and TT genotypes were protected against the development of CRC. An Italian population had similar results to the present study. This previous study found no significant effect of the G2677T polymorphism on the incidence of CRC or its prognosis (25). Another previous study conducted on Bulgarian patients indicated that the G2677T polymorphism is not a risk factor for CRC (12). Furthermore, other previous studies have demonstrated that there is no association between the *MDR1* G2677T polymorphism and the risk of CRC (10,11). However, numerous previous studies have also reported an association between this polymorphism and an increased CRC

risk (8,11,15,26). The conflicting results from these studies indicated that the silencing of Pgp expression may be due to exposure to environmental factors and, therefore, increased risk of malignancy. However, a causal relationship between *MDR1* and tumorigenesis has not been fully established. A previous study utilizing hybrid tagging and functional approaches has reported that *MDR1* polymorphisms served a minor role in the susceptibility to CRC development (9). Therefore, genetic-based studies are crucial in identifying biomarkers related to geographical, economic and pathological factors in a given population with a variety of ethnic features.

CEA a fetal glycoprotein that is considered to be a tumor marker that is widely used to clinically evaluate the responses of patients to treatment and tumor prognosis (20). The normal level of CEA is <2.5 ng/ml in adult non-smokers and ≤5 ng/ml in adult smokers (20). Previous empirical studies and meta-analyses have found that measuring CEA levels can aid in monitoring chemotherapeutic responses in patients with CRC, particularly when imaging protocols are unsuitable for assessing these responses in clinical practice (27,28). Additionally, measuring CEA levels can assist in determining the prognosis of patients with metastatic CRC receiving chemotherapy (28). However, measuring CEA levels alone has been demonstrated to be insufficient in improving survival rates (27). The present study examined patient responses to chemotherapy. The results of the current study demonstrated that neither of the two studied SNPs (T1236C and G2677T) in the *MDR1* gene were associated with the risk of chemoresistance to the currently used drug treatments XELIRI and XELOX (Table IV). Consistent with the results of the present study, a Korean study previously reported a non-significant association between the SNPs C3435T, G2677T and T1236C in the *MDR1* gene and drug resistance among Korean patients with epilepsy (29). A previous study conducted on Romanian pediatric patients indicated that the T1236C and G2677T polymorphisms of the *MDR1* gene were not associated with drug resistance in patients with epilepsy (30). However, the genotypes T1236T, T1236C and T2677T were found to be associated with drug-responsive idiopathic epilepsy (30). Furthermore, another previous study concluded that the T1236C polymorphism in the *MDR1* gene was significantly associated with a little to no response to therapy in patients with breast cancer in an Arab population of Saudi Arabia (31). The findings of another previous study demonstrated that the T1236C polymorphism was associated with drug resistance among female patients with epilepsy in the Iranian population whereas the T129C polymorphism was not associated with drug resistance in Iranian patients with epilepsy (14). A meta-analysis study indicated that the *MDR1* SNP T1236C contributed to responses to chemotherapy for several cancers, including osteosarcoma and breast cancer (32).

The present study has numerous limitations, including the small sample size, which affected the possibility in performing certain statistical analyses, including survival curve analysis that could aid in determining the role of *MDR1* SNPs in chemoresistance. This test in particular was not performed in the current study since all participants were still alive and none withdrew from the present study. Therefore, further studies involving larger number samples and tissues are required to confirm the reliability of the results of the present study.

In conclusion, results from the current study did not demonstrate an association between SNP T1236C in the *MDR1* gene and the risk of CRC development. However, SNP G2677T served a highly significant role in protecting against the development of CRC in our patients according to OR result. Moreover, a non-significant result was determined between the two SNPs and the risk of chemoresistance. Therefore, these two SNPs cannot be used as molecular markers for predicting drug response in patients with CRC.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

ABAG designed the current study. AMAQ performed experimental work. SNA collected blood samples. HAAD performed experimental work, drafted the manuscript and revised critically the intellectual content. HMT, AAZ and AMA provided the samples and diagnosed patients. SSA helped perform experimental work, collected blood samples, and wrote the questionnaire and consent forms. UMO and EME assisted in statistical analysis and acquisition of data. All authors have read and approved the final manuscript.

**Ethics approval and consent to participate**

The present study was approved by the General Directorate of Health Affairs in Jeddah, Saudi Arabia (approval no. A00221). Written informed consent and questionnaires were obtained from all participants.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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