

TaqI and ApaI Variants of Vitamin D Receptor Gene Increase the Risk of Colorectal Cancer in a Saudi Population

Ayat B. Al-Ghafari^{1,2,3}, Khadijah S. Balamash¹, Huda A. Al Doghaither¹

¹Department of Biochemistry, Faculty of Science, King Abdulaziz University, ²Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, ³Cancer and Mutagenesis Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

Abstract

Background: Polymorphisms in the gene encoding the vitamin D receptor (VDR) affect the protective role of vitamin D against many types of cancers, including colorectal cancer (CRC).

Objective: The objective of this study was to assess the effect of four major polymorphisms of the VDR gene (*Apal*, *TaqI*, *BsmI* and *FokI*) on the risk of CRC in a Saudi population.

Materials and Methods: This case-control study recruited 132 CRC patients from the oncology clinics at King Abdulaziz University Hospital and 124 healthy controls from the blood bank at King Fahad General Hospital, Jeddah, Saudi Arabia, between September 2017 and August 2018. All participants were Saudis and aged 20–80 years. Genomic DNA samples were extracted from the peripheral blood cells and amplified with polymerase chain reaction. The resulting fragments were digested with different endonucleases to reveal the genotypes using the restriction fragment length polymorphism technique. The genotype distribution and allele frequency, odds ratio (OR), risk ratio (RR) and *P* values were determined with contingency table analysis following Hardy–Weinberg equilibrium equation.

Results: For the *Apal* single-nucleotide polymorphism (SNP) (rs7975232), only the heterozygous (Aa) genotype increased the risk of CRC (OR = 3.4, RR = 2.3, and *P* < 0.0001), whereas the *TaqI* SNP (rs731236) carriers with either the heterozygous (Tt) or homozygous (tt) genotype displayed an increased risk for the disease (OR = 6.18, RR = 4, *P* < 0.0001; OR = 3, RR = 2.4, *P* = 0.02, respectively). In contrast, heterozygous (Bb) and homozygous (bb) carriers of the *BsmI* SNP (rs1544410) had significantly lower risk for CRC (*P* < 0.0001). Finally, for the *FokI* SNP (rs2228570), there was no association with CRC risk.

Conclusion: This study found that VDR SNPs *Apal* and *TaqI* increase the risk of CRC, whereas *BsmI* reduces the risk of CRC in the selected Saudi population. Therefore, *Apal* and *TaqI* SNPs could potentially be used as a diagnostic biomarker for CRC. However, the molecular mechanisms by which these variants increase or decrease the risk of CRC need to be investigated.

Keywords: *Apal*, colorectal cancer, Saudi, single-nucleotide polymorphism, *TaqI*, vitamin D receptor gene

Address for correspondence: Dr. Ayat B. Al-Ghafari, Department of Biochemistry, Faculty of Science, King Abdulaziz University, P O Box 8795, Jeddah 23817, Saudi Arabia.

E-mail: abalghafari@kau.edu.sa

Submitted: 17-Sep-2019 Revised: 21-Oct-2019 Accepted: 05-Jan-2020 Published: 20-Aug-2020

Access this article online

Quick Response Code:



Website:

www.sjmms.net

DOI:

10.4103/sjmms.sjmms_357_19

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Al-Ghafari AB, Balamash KS, Al Doghaither HA. *TaqI* and *Apal* variants of vitamin D receptor gene increase the risk of colorectal cancer in a Saudi population. Saudi J Med Med Sci 2020;8:188-95.

INTRODUCTION

Colorectal cancer (CRC) is a common cancer with significant variation in incidence rates worldwide. The lowest rates of CRC have been reported in South-Central Asia (3% for males and 2% for females), Central Africa (3% for males and 2.5% for females) and Central America (8.3% for males and 7.3% for females), whereas the highest rates are in North America (17.7% for males and 14.8% for females) and Southern Europe (25.4% for males and 15.9% for females).^[1] In Saudi Arabia, CRC accounted for 12.2% of all cancers in 2015 and was the second most common cancer type after breast cancer; it was the most common cancer in males (14.9%) and the third most common for females (9.9%).^[2] In contrast to several types of cancer, CRC most commonly occurs sporadically.^[3] Among Saudis, it presents at a younger age compared with Westerners.^[4] Although the underlying etiology of CRC is not well understood, it has been proposed that CRC results from an interaction between genetic, environmental and lifestyle factors.^[5-7]

Treatment of CRC has advanced over the past several years with the introduction of effective chemotherapeutic agents. In patients with advanced stages of cancer, chemotherapeutic drugs are often used in combination with biological therapy, such as minerals and vitamins, to enhance the response of the immune system.^[8] The biologically active form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), plays a crucial role in calcium homeostasis by enhancing the intestinal absorption of calcium and its reabsorption in the kidneys, which supports bone mineralization.^[9] In addition, vitamin D also has important nonskeletal functions, such as modulating the response of the innate and adaptive immune system^[10,11] and controlling other cellular processes, including proliferation, differentiation, apoptosis, invasion and angiogenesis.^[12,13]

Vitamin D exerts its biological effects through the transcription factor vitamin D receptor (VDR), which is a member of the nuclear receptor superfamily that also contains other receptors of medically important nuclear hormones, such as estrogen, testosterone and cortisol.^[12] The VDR gene is located on chromosome 12q12-14 and contains two promoter regions and eight exons.^[14,15] To date, >60 single-nucleotide polymorphisms (SNPs) of the VDR gene have been described. However, the most studied VDR SNPs, which showed potential association with many diseases and are in different regions of VDR gene are *BsmI* and *ApaI* (intron 8), *FokI* (exon 2), *TaqI* (exon 9), and poly (A) microsatellite (in the 3'-untranslated region).^[16,17] The role of VDR polymorphisms in the

development and prognosis of CRC has been extensively investigated, but contradictory results have been found in different populations.^[18-21] This is likely because VDR SNPs have different genotype distribution rates across different ethnic groups owing to the nature of interactions between these SNPs and other factors such as calcium and vitamin D intake, plasma level of 1,25(OH)₂D₃, ultraviolet-B exposure, obesity and diet.^[22-25] Therefore, identification of biomarkers based on the patient genetic map might act as a promising diagnostic approach for CRC either alone or in correlation with other environmental and clinical investigations.

In Saudi Arabia, to the best of the authors' knowledge, only one study has previously assessed the association of different genotypes of VDR gene and CRC.^[18] Additional analysis of gene polymorphisms in this population could lead to a better understanding of the role of VDR gene polymorphisms in the etiology. The aim of this study was to assess the association of different genotypes of VDR gene *ApaI*, *TaqI*, *BsmI* and *FokI* SNPs and the risk of developing CRC in Saudi patients who routinely visit the oncology clinics at King Abdulaziz University Hospital (KAUH) in Jeddah, Western region of Saudi Arabia.

MATERIALS AND METHODS

Materials and kits

A QIAamp DNA Blood Mini (250) Kit (catalog number: 51106) was purchased from Qiagen (Valencia, CA, USA). HotStarTaq Master Mix and RNase-free water (catalog number: 203601) were obtained from Affymetrix (Santa Clara, CA, USA). FastDigest restriction enzymes *ApaI* (catalog number: FD1414), *TaqI* (catalog number: FD0674), *MvaI269I* (catalog number: FD0964) and *FokI* (catalog number: FD2144) were all purchased from Thermo Fisher Scientific (Waltham, MA, USA). GeneRuler 100 bp DNA ladder (catalog number: SM0243) was also purchased from Thermo Fisher Scientific (Waltham, MA, USA).

Study design and participants

This case-control study recruited adult Saudi participants between September 2017 and August 2018. The study participants in both groups were chosen following specific inclusion and exclusion criteria. The CRC patients were recruited from the oncology clinics at KAUH, Jeddah, Saudi Arabia. Those participants were included if they had a biopsy- or colonoscopy-confirmed neoplasm; well-documented personal history, clinical history and tumor pathology data and availability of high-quality DNA extracted from blood sample. The patients were clinically classified according to the TNM Classification of Malignant

Tumors into Stages I and II (low-grade tumors) and Stages III and IV (high-grades tumor). Regarding chemotherapy treatment, Stage I patients did not receive any chemotherapy treatment, whereas patients in Stages II and III received chemotherapy either as XELIRI (intravenous irinotecan and capecitabine tablets), which lasted for 2–3 weeks (cycles), or XELOX (intravenous oxaliplatin and capecitabine tablets), which lasted for 3 weeks. Stage IV patients with metastatic tumor, mostly to liver, received a combination of XELIRI, XELOX, XELODA (capecitabine tablets, which lasted for 3 weeks [cycles]) and bevacizumab. CRC patients who were non-Saudis and had CRC as a secondary tumor were not included.

The healthy controls were recruited from the blood bank unit at King Fahad General Hospital in Jeddah, Saudi Arabia. For the control group, the study included individuals with a good overall health status and no family history of cancer. The selection was based on their clinical examination and laboratory investigations, especially carcinoembryonic antigen tumor marker levels. Those who were non-Saudi, had metabolic syndromes and/or chronic diseases were not included in the study.

The Biomedical Ethics Unit of the Faculty of Medicine at King Abdulaziz University approved this study, and it was conducted according to the principles of the Declaration of Helsinki, 2013, with regard to the patients' information, samples and results. The purpose of the study was explained to each participant and written and signed consent form was obtained before collecting blood samples.

Whole blood samples of all participants were drawn into lavender-top vacutainers containing anticoagulant (ethylenediaminetetraacetic acid), obtained from the oncology clinics at KAUH and the blood bank at King Fahad General Hospital in Jeddah, Saudi Arabia. Before proceeding with the genetic analysis of *VDR* variants, the collected blood samples of all included participants (both case and control) were investigated for the presence of major genetic mutations related to CRC using a CRC mutations panel at the Centre of Excellence in Genomic Medicine Research at King Abdulaziz University, Jeddah, Saudi Arabia. Participants in the control group whose samples did not express the wild genotype of the major oncogenes and tumor suppressor genes related to CRC development were excluded from the study.

DNA sample extraction and quality control

Genomic DNA samples were extracted from whole blood samples following the manufacturer's instructions for the QIAamp DNA Blood Mini (250) Kit. The DNA

concentration along with the purity of each extracted DNA sample was determined using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) with standard A260/A280 and A260/A230 ratios. All DNA samples were stored immediately after extraction at -20°C for later use.

Amplification of vitamin D receptor gene polymorphisms

Polymerase chain reaction (PCR) was performed in a final volume of 25 μl containing 2 μl of genomic DNA (100 ng/ μl), 12.5 μl of HotStarTaq Master Mix, 8.5 μl of RNase-free water and 1 μl of each forward and reverse primers. The primers and thermocycling conditions used to amplify different regions of the *VDR* gene are described in Table 1.

Determination of the different genotypes in the *VDR* gene

Restriction fragment length polymorphism (RFLP) was performed on all PCR products to determine the different genotypes of the *VDR* gene using four different enzymes. According to the manufacturer's instructions, the PCR product (10 μl), RNase-free water (17 μl), 10 \times FastDigest Green buffer (2 μl) and a specific FastDigest enzyme (*ApaI*, *TaqI*, *MvaI*269I [*BsmI*] or *FokI*) (1 μl) were mixed and incubated at 37°C for 20 min without an inactivation step. The digestion products were visualized on 2% agarose gels. To confirm the results of RFLP, 10% of the PCR samples were selected randomly and sent for DNA sequencing to the Center of Excellence in Genomic Medicine Research at King Fahd Medical Research Center, King Abdulaziz University, Saudi Arabia.

Statistical analysis

All statistical analyses were performed using GraphPad Prism version 7.00 (San Diego, CA, USA). A chi-square test was used to compare the allele frequency and genotype distribution of each variant with those expected for a population in Hardy–Weinberg equilibrium. The odds ratio (OR), risk ratio (RR) at 95% confidence interval and *P* values were determined by 2×2 contingency table analysis to describe the strength of association using a logistic regression model. The allele frequency was calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. An unpaired *t* test (Mann–Whitney test) was used to calculate the mean and standard error of mean and *P* value differences in physical characteristics such as age, weight, height, body mass index (BMI), waist circumference, hip circumference and waist-to-hip ratio (WHR) between CRC patients and controls. A multivariate regression model was applied to measure the correlation between a physical dependent variable (as mentioned) on the outcome (i.e., risk

of CRC development). $P < 0.05$ was considered statistically significant.

RESULTS

Demographic characteristics of the participants

A total of 170 controls were initially included in the study; however, the samples of 46 patients did not express the wild genotype of the major oncogenes and tumor suppressor genes related to CRC development, and thus were excluded. In the final analysis, 256 participants (CRC group = 132; controls = 124) aged 20–80 years were included. The mean age for the CRC group and controls were 55.73 ± 1.065 years and 40.69 ± 0.759 years, respectively. In the CRC group, there were 100 males and 32 females, while in the control group, there were 104 males and 20 females.

The anthropometric measurements of the participants are illustrated in Table 2. Compared with the CRC group, the healthy control group had significantly higher height ($P = 0.02$), age, weight and BMI (for all, $P < 0.0001$). To correlate these findings with the risk of CRC, a multivariate regression analysis was performed. Although the multivariate regression analysis revealed a significant difference in age ($P = 0.002$) between the two groups, this difference had no significant risk for cancer occurrence (OR = 1.072). In contrast, weight and BMI were not found to be significantly correlated with the risk of cancer occurrence ($P = 0.942$ and 0.463 and OR = 1.009 and 0.790, respectively). On the other hand, the other physical characteristics including hip and

waist circumferences and WHR showed a nonsignificant difference between the two groups ($P > 0.05$). In the CRC group, 28 had low-grade tumor, whereas 104 had high-grade tumor.

Genotype and allele frequencies of *ApaI* polymorphism

For the *ApaI* SNP (rs7975232), the genotypic frequencies of the patients were 45.5% ($n = 60$) for the normal genotype (AA), 37.9% ($n = 50$) for heterozygous genotype (Aa) and 16.7% ($n = 22$) for the homozygous genotype (aa). The frequencies of the “A” and “a” alleles were 64.39% and 35.61%, respectively. The genotype distribution for CRC patients was not in Hardy–Weinberg equilibrium (goodness-of-fit $\chi^2 = 3.99$, degree of freedom [DF] = 1, chi-square distribution table $P < 0.05$). The respective frequencies for the controls were 66.1% ($n = 82$) for normal genotype (AA), 16.1% ($n = 20$) for heterozygous genotype (Aa) and 17.7% ($n = 22$) for homozygous genotype (aa). The frequencies of the “A” and “a” alleles were 74.2% and 25.8%, respectively. The genotype distribution for the controls was not in Hardy–Weinberg equilibrium (goodness-of-fit $\chi^2 = 41.58$, DF = 1, $P < 0.05$) [Table 3]. A comparison of the results of RR and OR indicated that having a heterozygous genotype (Aa) significantly increased the risk of developing CRC compared to the normal genotype (AA) ($P < 0.0001$), whereas the homozygous genotype (aa) had no association with this risk ($P = 0.4$).

Genotype and allele frequencies of *TaqI* polymorphism

For the *TaqI* SNP (rs731236), the genotypic frequencies of the patients were 50.0% ($n = 66$) for normal

Table 1: Sequence of forward (For) and reverse (Rev) polymerase chain reaction primers and thermocycler conditions used for amplification of vitamin D receptor gene polymorphisms

| Locus | Primers (5'-3') | Thermocycling conditions | Reference |
|-------------|---|--|-----------|
| <i>ApaI</i> | For: ggatcctaaatgcacggaga Rev: acgtctgcagtggtggac | 95°C/5 min, (95°C/1 min, 55°C/1 min, 72°C/1 min) × 35, 72°C/10 min | [22] |
| <i>TaqI</i> | For: cagagcatggacaggagcaag Rev: gcaactcctcatgggctgaggtctca | 94°C/4 min, (94°C/1 min, 64°C/1 min, 72°C/1 min) × 35, 72°C/7 min | [28] |
| <i>BsmI</i> | For: cctcactgcccttagctctg Rev: tgcctcaaaaatcaatcagg | 95°C/5 min, (95°C/1 min, 55°C/1 min, 72°C/1 min) × 35, 72°C/10 min | [22] |
| <i>FokI</i> | For: gatccagctggccctggcactg Rev: atggaaacactgtcttcttccctc | 94°C/4 min, (94°C/1 min, 60°C/1 min, 72°C/1 min) × 35, 72°C/4 min | [28] |

Table 2: Physical comparisons between Saudi colorectal cancer patients and control groups

| Physical characteristics | Mean±SEM | | Unpaired <i>t</i> -test <i>P</i> |
|--------------------------|---------------------------|---------------------------|----------------------------------|
| | Patients (<i>n</i> =132) | Controls (<i>n</i> =124) | |
| Age (years) | 55.73±1.065 | 40.69±0.759 | <0.0001 |
| Height (cm) | 165.1±0.809 | 168.0±0.9291 | 0.02 |
| Weight (kg) | 73.17±1.363 | 83.85±1.429 | <0.0001 |
| Hip circumference (cm) | 110.0±1.623 | 108.6±1.626 | 0.53 |
| Waist circumference (cm) | 100.3±1.715 | 101.9±1.729 | 0.52 |
| WHR | 0.9174±0.012 | 0.9446±0.012 | 0.11 |
| BMI (kg/m ²) | 26.84±0.489 | 29.85±0.495 | <0.0001 |

SEM – Standard error of mean; WHR – Waist-to-hip ratio; BMI – Body mass index

genotype (TT), 36.4% ($n = 48$) for heterozygous genotype (Tt) and 13.6% ($n = 18$) for homozygous genotype (tt) genotypes. The frequencies of the “T” and “t” alleles were 68.2% and 31.8%, respectively. The genotype distribution for CRC patients was in Hardy–Weinberg equilibrium (goodness-of-fit $\chi^2 = 3.451$, DF = 1, $P > 0.05$). In controls, 82.3% ($n = 102$) were normal genotype (TT), 9.7% ($n = 12$) were heterozygous genotype (Tt) and 8.1% ($n = 10$) were homozygous genotype (tt). The frequencies of the “T” and “t” alleles were 87.1% and 12.9%, respectively. The genotype distribution for the controls was not in Hardy–Weinberg equilibrium (goodness-of-fit $\chi^2 = 40.31$, DF = 1, $P < 0.05$) [Table 4]. A comparison of the results for RR and OR showed that both the heterozygous (Tt) and homozygous (tt) genotypes had significantly increased risk of developing CRC compared to the normal genotype (TT) ($P < 0.0001$ and $P = 0.02$, respectively). Therefore, the replacement of the major allele “T” with the minor allele “t,” either in the form of major or recessive genotypes, might increase the risk of developing CRC ($P = 0.002$).

Genotype and allele frequencies of BsmI polymorphism

For the *BsmI* SNP (rs1544410), the genotypic frequencies of the patients were 37.9% ($n = 50$) for the normal genotype (BB), 43.9% ($n = 58$) for the heterozygous genotype (Bb) and 18.2% ($n = 24$) for the homozygous genotype (bb). The frequencies of the “B” and “b” alleles were 59.9% and 40.1%, respectively. The genotype distribution for CRC patients was in Hardy–Weinberg equilibrium (goodness-of-fit $\chi^2 = 0.971$, DF = 1, $P > 0.05$). In controls, 6.5% ($n = 8$) were normal genotype (BB), 57.2% ($n = 71$) heterozygous genotype (Bb) and

36.3% ($n = 45$) homozygous genotype (bb). The frequencies of the “B” and “b” alleles were 35.1% and 64.9%, respectively. The genotype distribution for the controls was not in Hardy–Weinberg equilibrium (goodness-of-fit $\chi^2 = 8.196$, DF = 1, $P < 0.001$) [Table 5]. The results of RR and OR indicated that both the heterozygous (Bb) and homozygous (bb) genotypes decreased the risk of developing CRC compared to the normal genotype (BB) ($P < 0.0001$).

Genotype and allele frequencies of FokI polymorphism

For the *FokI* SNP (rs2228570), the genotypic frequencies of the patients were 63.6% ($n = 84$) for normal genotype (FF), 27.3% ($n = 36$) for heterozygous genotype (Ff) and 9.1% ($n = 12$) for homozygous genotype (ff). The frequencies of the “F” and “f” alleles were 77.3% and 22.7%, respectively. The genotype distribution for CRC patients was not in Hardy–Weinberg equilibrium (goodness-of-fit $\chi^2 = 6.61$, DF = 1, $P < 0.01$). The controls were 62.1% ($n = 77$) normal genotype (FF), 29.8% ($n = 37$) heterozygous genotype (Ff) and 8.1% ($n = 10$) homozygous genotype (ff). The frequencies of the “F” and “f” alleles were 77.0% and 23.0%, respectively. The genotype distribution for the controls was in Hardy–Weinberg equilibrium (goodness-of-fit $\chi^2 = 3.059$, DF = 1, $P > 0.05$) [Table 6]. A comparison of the results of RR and OR showed that both the heterozygous (Ff) and homozygous (ff) genotypes had no significant correlation with the risk of developing CRC compared to normal genotype (FF) ($P = 1$).

DISCUSSION

In the present study population, of the four major polymorphisms of the *VDR* gene for which the genotype

Table 3: Distribution of genotypes and alleles frequency of the *Apal* polymorphism of the vitamin D receptor gene among colorectal cancer patients and controls

| <i>Apal</i> polymorphism | Frequencies (%) | | Fisher's <i>P</i> | OR (95% CI) | RR (95% CI) |
|--------------------------|----------------------|----------------------|-------------------|---------------------|-----------------------|
| | Patients ($n=132$) | Controls ($n=124$) | | | |
| Wild type (AA) | 45.45% ($n=60$) | 66.13% ($n=82$) | | 1.00 (reference) | 1.00 (reference) |
| Heterozygous (Aa) | 37.88% ($n=50$) | 16.13% ($n=20$) | <0.0001 | 3.417 (1.845-6.327) | 2.318 (1.4884-3.6106) |
| Homozygous (aa) | 16.67% ($n=22$) | 17.74% ($n=22$) | 0.4 | 1.367 (0.694-2.693) | 1.268 (0.758-2.123) |
| Dominant allele (A) | 64.39% | 74.20% | | 1.00 (reference) | 1.00 (reference) |
| Recessive allele (a) | 35.61% | 25.80% | 0.2 | 1.601 (0.874-2.933) | 1.385 (0.908-2.110) |

OR – Odds ratio; CI – Confidence interval

Table 4: Distribution of genotypes and alleles frequency of the *TaqI* polymorphism of the vitamin D receptor gene among colorectal cancer patients and controls

| <i>TaqI</i> polymorphism | Frequencies (%) | | Fisher's <i>P</i> | OR (95% CI) | RR (95% CI) |
|--------------------------|----------------------|----------------------|-------------------|--------------------|-------------------|
| | Patients ($n=132$) | Controls ($n=124$) | | | |
| Wild type (TT) | 50% ($n=66$) | 82.26% ($n=102$) | | 1.00 (reference) | 1.00 (reference) |
| Heterozygous (Tt) | 36.36% ($n=48$) | 9.68% ($n=12$) | <0.0001 | 6.2 (3.057-12.502) | 4 (2.247-7.122) |
| Homozygous (tt) | 13.64% ($n=18$) | 8.06% ($n=10$) | 0.02 | 3 (1.209-6.397) | 2.4 (1.169-4.298) |
| Dominant allele (T) | 68.18% | 87.10% | | 1.00 (reference) | 1.00 (reference) |
| Recessive allele (t) | 31.82% | 12.90% | 0.002 | 3 (1.535-6.460) | 2.5 (1.376-4.405) |

OR – Odds ratio; CI – Confidence interval

Table 5: Distribution of genotypes and alleles frequency of the *BsmI* polymorphism of the vitamin D receptor gene among colorectal cancer patients and controls

| <i>BsmI</i> polymorphism | Frequencies (%) | | Fisher's P | OR (95% CI) | RR (95% CI) |
|--------------------------|------------------|------------------|------------|---------------------|---------------------|
| | Patients (n=132) | Controls (n=124) | | | |
| Wild type (BB) | 37.88% (n=50) | 6.45% (n=8) | | 1.00 (reference) | 1.00 (reference) |
| Heterozygous (Bb) | 43.94% (n=58) | 57.26% (n=71) | <0.0001 | 0.131 (0.057-0.298) | 0.598 (0.494-0.723) |
| Homozygous (bb) | 18.18% (n=24) | 36.29% (n=45) | <0.0001 | 0.085 (0.035-0.209) | 0.382 (0.269-0.541) |
| Dominant allele (B) | 59.85% | 35.08% | | 1.00 (reference) | 1.00 (reference) |
| Recessive allele (b) | 40.15% | 64.92% | <0.0001 | 0.359 (0.202-0.637) | 0.615 (0.465-0.814) |

OR – Odds ratio; CI – Confidence interval

Table 6: Distribution of genotypes and alleles frequency of the *FokI* polymorphism of the vitamin D receptor gene among colorectal cancer patients and controls

| <i>FokI</i> polymorphism | Frequencies (%) | | Fisher's P | OR (95% CI) | RR (95% CI) |
|--------------------------|------------------|------------------|------------|---------------------|----------------------|
| | Patients (n=132) | Controls (n=124) | | | |
| Wild type (FF) | 63.64% (n=84) | 62.10% (n=77) | | 1.00 (reference) | 1.00 (reference) |
| Heterozygous (Ff) | 27.27% (n=36) | 29.84% (n=37) | 0.77 | 0.8919 (0.513-1.55) | 0.9243 (0.632-1.352) |
| Homozygous (ff) | 9.09% (n=12) | 8.06% (n=10) | 1 | 1.1 (0.449-2.690) | 1.09 (0.495-2.390) |
| Dominant allele (F) | 77.28% | 77.02% | | 1.00 (reference) | 1.00 (reference) |
| Recessive allele (f) | 22.72% | 22.98% | 1 | 1 (0.518-1.932) | 1 (0.602-1.661) |

OR – Odds ratio; CI – Confidence interval

distribution and allele frequency analysis was carried out in CRC patients and healthy controls, only the heterozygous genotype (Aa) of SNP *ApaI* and the heterozygous (Tt) and homozygous (tt) genotypes of SNP *TaqI* were found to significantly increase the risk of CRC. In contrast, the heterozygous (Bb) and homozygous (bb) genotypes of SNP *BsmI* significantly lowered the risk of developing CRC. The genotypes of SNP *FokI* were not associated with either the prognosis of or protection from CRC in the study participants.

Some SNPs in the study deviated from Hardy–Weinberg equilibrium, which can be explained by many factors such as the small sample size of the study that may have resulted in genetic drift, a likely gene flow exists between generations (i.e., it is not preserved), mutations or SNPs in the *VDR* gene exist in the population, individuals were chosen randomly and natural selection was not operating on the study population. Regardless of these findings, although *VDR*-associated SNPs have been extensively studied previously, their general association with tumorigenesis remains controversial based on several factors such as age, sex, intake of vitamin D and other supplements, stage of cancer and ethnicity.^[26] Regarding CRC, most studies on *VDR* SNPs have shown an association between the genotype of SNPs *FokI*, *BsmI* and *TaqI*, but not with *ApaI*. In contrast to our results, Laczanska *et al.*^[27] found that Polish CRC patients with the homozygous genotype (bb) of the SNP *BsmI* were more susceptible to CRC compared with those with other genotypes. They also reported that patients with the *TaqI* normal genotype (TT) and *ApaI* normal genotype (AA) had a higher risk of CRC. A study performed on 100

Saudi CRC patients in 2016 showed a nonsignificant association for these four *VDR* polymorphisms and the CRC risk. However, in agreement with the current results, patients with a normal *BsmI* genotype were protected against CRC compared to the homozygous genotype carriers.^[18] In Egyptian CRC patients, the SNP *FokI* was nonsignificant associated with CRC progression, which is similar to our findings, whereas the SNPs *ApaI* and *TaqI* were not significantly correlated with CRC progression and transformation, which is in contrast to our results.^[28] In addition, the homozygous genotype of the *BsmI* SNP is associated with a decreased risk of breast cancer in Latina women.^[29] Recently, in contrast to the current study results, a meta-analysis that included 39 articles of five *VDR* gene polymorphisms, namely, *ApaI*, *FokI*, *BsmI*, *TaqI* and *Cdx 2*, and assessed their association with CRC found that *BsmI* is associated with CRC risk and *FokI* may be a risk factor for CRC.^[30]

To the best of the authors' knowledge, this is only the second study from Saudi Arabia that has assessed *VDR* SNPs and its association with CRC development.^[18] Compared with the other study, the current study had a larger sample size and found more correlations between the SNPs and CRC, highlighting the strength of the current study. As a limitation, it should be noted that Jeddah is more likely to have a higher diverse gene pool among Saudis due to larger rates of nonconsanguineous marriage compared with some other regions, where the rates of consanguineous marriages are higher. Therefore, this may limit the generalizability of the current study findings. In addition, the ratio between the numbers of CRC patients to healthy control in the current study was 1:0.94, which is slightly lower than the ideal ratio

in case-control studies and this may slightly affect the precision level of some statistical analyses. Moreover, the nonmatching for age between CRC patients and controls needs to be considered when considering the results of the current study. A larger sample size from a wider area would increase the generalizability of the study results, and thus a large-scale, multicenter study from across Saudi Arabia is needed for better understanding of the role of VDR gene polymorphisms in CRC. Although some SNPs showed a deviation from Hardy-Weinberg equation, which might be because of the population size, inherent bias of the Hardy-Weinberg equation disequilibrium and other patient recruitment characteristics, this is unlikely to have any serious implications on the study results. However, these aspects need to be investigated further in future studies.

CONCLUSION

This study showed that the *TaqI* and *ApaI* SNPs increased the risk of CRC in the studied population, whereas the *BsmI* SNP confers protective effect. However, the molecular mechanisms by which these variants increase or decrease the risk of CRC needs to be investigated.

Ethical considerations

This study was conducted after obtaining the ethical approval from the Biomedical Ethics Unit of the Faculty of Medicine at King Abdulaziz University (Reference no. 379-17) on August 8, 2017, and in accordance with the principles of the Declaration of Helsinki, 2013. All participants provided a signed written informed consent before inclusion in the study.

Peer review

This article was peer-reviewed by two independent and anonymous reviewers.

Acknowledgments

This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant no. RG-6-130-38. The authors, therefore, acknowledge with thanks DSR for their technical and financial support. The authors would like also to express their thanks to Professor Carsten Carlberg, Professor of Biochemistry at University of Eastern Finland, for his critical contribution in reviewing the manuscript.

Financial support and sponsorship

This project was funded by the Deanship of Scientific Research, King Abdulaziz University, Jeddah, under grant no. (RG-6-130-38).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
- National Health Information Center at the Saudi Cancer Registry. Cancer Incidence Report. Riyadh, Kingdom of Saudi Arabia: National Health Information Center at the Saudi Cancer Registry; 2015.
- Shaik AP, Shaik AS, Al-Sheikh YA. Colorectal cancer: A review of the genome-wide association studies in the Kingdom of Saudi Arabia. *Saudi J Gastroenterol* 2015;21:123-8.
- Zubaidi AM, AlSubaie NM, AlHumaid AA, Shaik SA, AlKhayal KA, AlObeed OA. Public awareness of colorectal cancer in Saudi Arabia: A survey of 1070 participants in Riyadh. *Saudi J Gastroenterol* 2015;21:78-83.
- Ma H, Brosens LA, Offerhaus GJ, Giardiello FM, de Leng WW, Montgomery EA. Pathology and genetics of hereditary colorectal cancer. *Pathology* 2018;50:49-59.
- Granados-Romero JJ, Valderrama-Treviño AI, Contreras-Flores EH, Barrera-Mera B, Enríquez MH, Uriarte-Ruiz K, et al. Colorectal cancer: A review. *Int J Res Med Sci* 2017;5:4667-76.
- Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, et al. Colorectal cancer. *Nat Rev Dis Primers* 2015;1:15065.
- Carethers JM. Systemic treatment of advanced colorectal cancer: Tailoring therapy to the tumor. *Therap Adv Gastroenterol* 2008;1:33-42.
- Fleet JC. The role of vitamin D in the endocrinology controlling calcium homeostasis. *Mol Cell Endocrinol* 2017;453:36-45.
- Chirumbolo S, Bjørklund G, Sboarina A, Vella A. The role of vitamin D in the immune system as a pro-survival molecule. *Clin Ther* 2017;39:894-916.
- Dou R, Ng K, Giovannucci EL, Manson JE, Qian ZR, Ogino S. Vitamin D and colorectal cancer: Molecular, epidemiological and clinical evidence. *Br J Nutr* 2016;115:1643-60.
- Belorusova AY, Rochel N. Structural studies of vitamin D nuclear receptor ligand-binding properties. *Vitam Horm* 2016;100:83-116.
- Ma Y, Johnson CS, Trump DL. Mechanistic insights of vitamin D anticancer effects. *Vitam Horm* 2016;100:395-431.
- Miyamoto K, Kesterson RA, Yamamoto H, Taketani Y, Nishiwaki E, Tatsumi S, et al. Structural organization of the human vitamin D receptor chromosomal gene and its promoter. *Mol Endocrinol* 1997;11:1165-79.
- Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ, Haussler MR, et al. Cloning and expression of full-length cDNA encoding human vitamin D receptor. *Proc Natl Acad Sci U S A* 1988;85:3294-8.
- Köstner K, Denzer N, Müller CS, Klein R, Tilgen W, Reichrath J. The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: A review of the literature. *Anticancer Res* 2009;29:3511-36.
- Vuolo L, Di Somma C, Faggiano A, Colao A. Vitamin D and cancer. *Front Endocrinol (Lausanne)* 2012;3:58.
- Alkhayal KA, Awadalia ZH, Vaali-Mohammed MA, Al Obeed OA, Al Wesaimer A, Halwani R, et al. Association of vitamin D receptor gene polymorphisms with colorectal cancer in a Saudi Arabian population. *PLoS One* 2016;11:e0155236.
- Budhathoki S, Yamaji T, Iwasaki M, Sawada N, Shimazu T, Sasazuki S, et al. Vitamin D receptor gene polymorphism and the risk of colorectal cancer: A nested case-control study. *PLoS One* 2016;11:e0164648.
- Vidigal V, da Silva T, Pimenta C, Oliveira J, Felipe A, Forones N. Genetic polymorphism of vitamin D receptor *BsmI*, *ApaI* and *CYP27B1*, *CYP24A1* genes and the risk of colorectal cancer. *Ann*

- Oncol 2015;26:iv1-100.
21. Sarkissyan M, Wu Y, Chen Z, Mishra DK, Sarkissyan S, Giannikopoulos I, *et al.* Vitamin D receptor FokI gene polymorphisms may be associated with CRC among African American and Hispanic participants. *Cancer* 2014;120:1387-93.
 22. Hajj A, Chedid R, Chouery E, Megarbané A, Gannagé-Yared MH. Relationship between vitamin D receptor gene polymorphisms, cardiovascular risk factors and adiponectin in a healthy young population. *Pharmacogenomics* 2016;17:1675-86.
 23. Morales-Oyarvide V, Meyerhardt JA, Ng K. Vitamin D and physical activity in patients with colorectal cancer: Epidemiological evidence and therapeutic implications. *Cancer J* 2016;22:223-31.
 24. John EM, Schwartz GG, Koo J, Wang W, Ingles SA. Sun exposure, vitamin D receptor gene polymorphisms, and breast cancer risk in a multiethnic population. *Am J Epidemiol* 2007;166:1409-19.
 25. John EM, Schwartz GG, Koo J, van Den Berg D, Ingles SA. Sun exposure, vitamin D receptor gene polymorphisms, and risk of advanced prostate cancer. *Cancer Res* 2005;65:5470-9.
 26. Rai V, Abdo J, Agrawal S, Agrawal DK. Vitamin D receptor polymorphism and cancer: An update. *Anticancer Res* 2017;37:3991-4003.
 27. Laczmanska I, Laczmanski L, Bebenek M, Karpinski P, Czernarmazowicz H, Ramsey D, *et al.* Vitamin D receptor gene polymorphisms in relation to the risk of colorectal cancer in the Polish population. *Tumour Biol* 2014;35:12397-401.
 28. Rizk M, Zakaria N, Elshazely W. Study of vitamin D receptor (VDR) gene polymorphisms among Egyptian cohort patients with different stages of colorectal cancer. *J Cancer Ther* 2014;5:253-263.
 29. Ingles SA, Garcia DG, Wang W, Nieters A, Henderson BE, Kolonel LN, *et al.* Vitamin D receptor genotype and breast cancer in Latinas (United States). *Cancer Causes Control* 2000;11:25-30.
 30. Pan Z, Chen M, Hu X, Wang H, Yang J, Zhang C, *et al.* Associations between VDR gene polymorphisms and colorectal cancer susceptibility: An updated meta-analysis based on 39 case-control studies. *Oncotarget* 2018;9:13068-76.