Association between polymorphisms in \textit{PRNCR1} and risk of colorectal cancer in the Saudi population

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Abstract

LncRNA Prostate cancer non-coding RNA (\textit{PRNCR1}) is downregulated in many types of cancer. The current case-control study was performed on 144 patients with colorectal cancer and 130 matching controls. Genotyping was performed using TaqMan assays for four Single Nucleotide Polymorphisms (SNPs) in \textit{PRNCR1}. RNAsnp Web Server was used to detect variations in the secondary structure for each SNP. The genotyping analysis for SNP rs1456315 showed increased association with colorectal cancer with the homozygous CC variant allele (OR: 2.09; $\chi^2 = 4.95$; CI: 1.08–4.02; $p = 0.02$), the minor allele frequency, and additive genotype, respectively (OR: 1.55; $\chi^2 = 6.24$; CI: 1.09–2.19; $p = 0.01$) & (OR: 1.64; $\chi^2 = 4.04$; CI: 1.01–2.67; $p = 0.04$). A risk association was also observed among younger age patients (<57) and in female patients as well as in patients with tumors of the colon. For the other SNPs tested (rs16901946, rs13252298, rs1016343), no significant association was observed. The secondary structure of the rs1456315 mutant is different from that of the wild-type. Our findings suggest that the upregulation of \textit{PRNCR1} and its variants is associated with increased risk of colorectal cancer in Saudi patients, indicating that \textit{PRNCR1} might be a unique and valuable signature for predicting the risk of colorectal cancer in a Saudi population.

Introduction

Colorectal cancer develops due to the accumulation of a series of epigenetic and genetic variations. Several biological pathways may drive the development of epithelial cells into colorectal adenocarcinoma. The genetic basis for colorectal cancer is depicted as a multistep model of cancer development [1]. Long noncoding RNAs are described to be key genetic regulators of crucial biological processes that play roles in cancer and other diseases. In general, lncRNAs...
are RNA transcripts which are longer than 200 nucleotides but do not code for any proteins [2]. Some lncRNAs, such as Prostate cancer non-coding RNA 1 (PRNCR1), HOTAIR, and UCA1, are believed to be involved in cancer development. Long noncoding RNA PRNCR1 is downregulated in many types of cancer. PRNCR1 is a ~13 kb intron-less lncRNA which is transcribed from 8q24 [3]. Previous studies found that PRNCR1 plays a vital role in the development of prostate cancer predisposition and it might be pivotal in prostate cancer progression by modifying androgen receptor (AR) function. Recent studies demonstrated that PRNCR1 plays a critical role in progression of several cancers [4] [5]. Yang et al. (2013) reported that PRNCR1 enhances the chances of prostate cancer progression by changing the AR mechanism. PRNCR1 binds to the androgen receptor acetylated region and its relationship with DOT1L seems to be mandatory for recruitment of PCGEM to the DOT1L-mediated methylation of AR at the N-terminus. The collaborations of these overexpressed lncRNAs could hypothetically act as essential regulators in prostate cancer. However, the relationship between lncRNA PRNCR1 and development of colorectal cancer was previously unknown. In recent years many reports have stated that the variants of 8q24 may contribute to a predisposition for colon cancer [6]. PRNCR1 is situated in a sensitive region of the genomic area for CRC, yet the role of PRNCR1 is still not yet studied in Saudi populations for any cancer. Recent findings recommended that people with PRNCR1 variants may have a higher risk of developing cancer [7]. In the present study, we planned to evaluate the association of PRNCR1 variants with Saudi colorectal cancer progression. To achieve this goal, we sought to use genotyping and gene expression methods to evaluate the risk variants’ role and gene expression levels in Saudi colorectal cancer patients.

**Materials and methods**

**Sampling and DNA isolation**

A total of 144 colon cancer blood samples were collected from at endoscopy department of King Khalid University Hospital, King Saud University from 2012 to 2017 as per the guidelines of IRB. The study protocol was reviewed and approved by the local ethic committee from KKUH (Project approval Number E12-596), and all study patients gave their written informed consent. The diagnosis and confirmation of colorectal cancer were done based on endoscopy and histopathological results. Simultaneously, 130 healthy age- and sex-matched individuals were recruited as controls. The demographic and clinical data of the patients and controls are presented in Table 1. DNA was isolated from peripheral blood samples using the Qiagen blood DNA kit.

**Genotyping**

Based on previous reports, four SNPs, rs1016343 (C_7531214_10), rs13252298 (C_1645362_20), rs16901946 (C_33280498_20), and rs1456315 (C_7531200_20) in PRNCR1 were selected for the present study. Genotyping was performed using TaqMan genotyping assays (Applied biosystems, USA) in colorectal cancer (n = 144) and matched peripheral blood DNA samples (n = 130) (S1 and S2 Tables). Genotyping experiments were conducted using Quant Studio 7 (Applied Biosystems, USA) as described by Alanazi et al. [8].

**Statistical analyses**

A $\chi^2$ test was used to evaluate the demographic differences, variables, and genotypes of the PRNCR1 polymorphic variants; the Hardy-Weinberg equilibrium was tested by a $\chi^2$ adjustment test to compare the frequencies of the genotypes observed with the frequencies expected
among the controls. Odds ratios, confidence intervals, and significance were calculated using a web tool https://ihg.gsf.de/cgi-bin/hw/hwa1.pl. Bonferroni correction was used for multiple comparison of p-value. RNAsnp Web Server (https://rth.dk/resources/rnasnp/) [9], was used to predict the secondary structure for PRNCR1 variants with each SNP.

Results

The present case-control study comprised 144 colorectal cancer patients and 130 healthy subjects that closely matched the patients in terms of age and sex. The clinical data of the patients are presented in Table 1. The patients had a median age of 57 years (range 23–79 years) with 83 males and 61 females. A total of 91 patients had colon cancer while 53 patients had rectal cancer. All stages of CRC were included in the study: stage I (19 samples), stage II (50 samples), stage III (58 samples) and stage IV (17 samples). The healthy subjects had a median age of 57 years (range 23–79 years) with 71 males and 59 females.

Out of the four SNPs only one SNP, rs1456315, showed significant risk association with colorectal cancer. Table 3 shows the obtained genotype and allele frequencies and significance of the genotype and allele distribution of the tested SNPs. The results showed that PRNCR1 SNP rs1456315 had a statistically significant risk association with Saudi CRC patients. The homozygous variant “CC” genotype showed significant association (OR: 2.09; $\chi^2 = 4.95$; CI:

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Table 1. Clinical and demographic characteristics of the study subjects. Percentage of total cases are shown in parentheses.

| Clinical characteristic | CRC Cases | Controls |
|------------------------|-----------|----------|
|                        | Number    |          |
| Gender                 |           |          |
| Male                   | 83 (57.64)| 71 (54.62)|
| Female                 | 61 (42.36)| 59 (45.38)|
| Age                    |           |          |
| < 58                   | 66 (45.14)| 76 (58.46)|
| > 58                   | 78 (54.86)| 54 (41.54)|
| Tumor location         |           |          |
| Colon                  | 91 (63.19)|          |
| Rectum                 | 53 (36.81)|          |
| Tumor stage            |           |          |
| I                      | 19 (13.19)|          |
| II                     | 50 (34.72)|          |
| III                    | 58 (40.28)|          |
| IV                     | 17 (11.81)|          |

Table 2. Primary information for PRNCR1 polymorphisms.

| Genotyped SNPs | rs1016343 | rs13252298 | rs16901946 | rs1456315 |
|----------------|-----------|------------|------------|-----------|
| Chromosome     | 8         | 8          | 8          | 8         |
| Chr Pos        | 127081052 | 127082911  | 127088680  | 127091692 |
| Base change    | C>T       | A>G        | A>G        | T>C       |
| MAF in controls| 0.16      | 0.28       | 0.02       | 0.48      |
| P value for HWEb test in our controls | 0.54 | 1.0 | 1.0 | 0.08 |

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The minor allele "C" and additive genotype "TC+CC" also showed significant association (OR: 1.55; \( \chi^2 = 6.24; \) CI: 1.098–2.193; \( p = 0.01 \)) & (OR: 1.64; \( \chi^2 = 4.04; \) CI: 1.011–2.675; \( p = 0.04 \)), respectively. Only SNP rs1456315 minor allele C showed significant association even after Bonferroni correction (\( p = 0.04 \)). The remaining three SNPs rs1016343 (C>T), rs13252298 (A>G), and rs16901946 (A>G) did not show any association with colorectal cancer (Table 3).

To check the association of PRNCR1 SNPs with age, gender, and tumor location of the patients, we did a further stratified analysis. To assess the association of PRNCR1 SNPs with age, cancer and control samples were stratified into two groups split on median age as \( \leq 57 \) years, and \( >57 \) years. The genotype frequencies of both the groups are shown in Tables 4 and 5, respectively.

SNP rs1456315, which showed significant association in the overall analysis, showed significant risk association in patients below 57 years old. The SNP rs1456315 homozygous variant “CC” genotype showed a 3.3-fold more risk association (OR: 3.31; \( \chi^2 = 6.38; \) CI: 1.283–8.563; \( p = 0.01 \)), while "TC+CC" showed a 2.3-fold risk (OR: 2.37; \( \chi^2 = 6.08; \) CI: 1.186–4.741; \( p = 0.01 \)), even after Bonferroni correction (\( p = 0.04 \)), specifically in patients \( \leq 57 \) years of age. The minor allele “C” showed 2-fold significant association (OR: 2.03; \( \chi^2 = 8.31; \) CI: 1.252–3.31;

Table 3. Relationships of polymorphisms in PRNCR1 gene and colorectal cancer susceptibility. Percentage of total cases are shown in parentheses.

| SNP     | Variant | patients | Cases | Controls | OR     | CI    | \( \chi^2 \) Value | P- Value |
|---------|---------|----------|-------|----------|--------|-------|-------------------|----------|
| rs1456315 | TT      | 50(0.35) | 61(0.47) | Ref      |        |       |                   |          |
|         | TC      | 57(0.40) | 48(0.37) | 1.449    | 0.848–2.476 | 1.84  | 0.17458          |          |
|         | CC      | 36(0.25) | 21(0.16) | 2.091    | 1.086–4.028 | 4.95  | 0.02617*          |          |
|         | TC+CC   | 93(0.65) | 69(0.53) | 1.644    | 1.011–2.675 | 4.04  | 0.04455*          |          |
|         | T       | 157(0.55)| 170(0.65)| Ref      |        |       |                   |          |
|         | C       | 129(0.45)| 90(0.35) | 1.552    | 1.098–2.193 | 6.24  | 0.01250          |          |
| rs16901946 | AA     | 140(0.97)| 128(0.98)| Ref      |        |       |                   |          |
|         | AG      | 4(0.03)  | 2(0.02)  | 1.829    | 0.329–10.153 | 0.49  | 0.48396          |          |
|         | GG      | 0        | 0        | 0.915    | 0.018–46.430 | 0.01  | 1.00000          |          |
|         | AG+GG   | 4(0.03)  | 2(0.02)  | 1.829    | 0.329–10.153 | 0.49  | 0.48396          |          |
|         | A       | 284(0.99)| 258(0.99)| Ref      |        |       |                   |          |
|         | G       | 4(0.01)  | 2(0.01)  | 1.817    | 0.330–10.003 | 0.48  | 0.68981          |          |
| rs13252298 | AA     | 74(0.51) | 58(0.47) | Ref      |        |       |                   |          |
|         | AG      | 59(0.41) | 51(0.41) | 0.907    | 0.545–1.508 | 0.14  | 0.70586          |          |
|         | GG      | 11(0.08) | 14(0.11) | 0.616    | 0.260–1.457 | 1.23  | 0.26713          |          |
|         | AG+GG   | 70(0.49) | 65(0.53) | 0.844    | 0.521–1.367 | 0.48  | 0.49031          |          |
|         | A       | 207(0.72)| 167(0.68)| Ref      |        |       |                   |          |
|         | G       | 81(0.28) | 79(0.32) | 0.827    | 0.571–1.199 | 1.01  | 0.31588          |          |
| rs1016343 | CC     | 100(0.70)| 92(0.71) | Ref      |        |       |                   |          |
|         | CT      | 37(0.26) | 34(0.26) | 1.001    | 0.581–1.727 | 0.001 | 0.99663          |          |
|         | TT      | 5(0.04)  | 3(0.02)  | 1.533    | 0.356–6.596 | 0.33  | 0.56321          |          |
|         | CT+CC   | 42(0.30) | 37(0.29) | 1.044    | 0.618–1.765 | 0.03  | 0.87133          |          |
|         | C       | 237(0.83)| 218(0.84)| Ref      |        |       |                   |          |
|         | T       | 47(0.17) | 40(0.16) | 1.081    | 0.682–1.712 | 0.11  | 0.74055          |          |

* = not significant after Bonferroni correction

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Table 4. The association between PRNCR1 gene polymorphism and the risk of colorectal cancer in patients younger than 58 years. Percentage of total cases are shown in parentheses.

| SNP       | Variant | patients | Cases | Controls | OR      | CI      | χ² Value | P- Value |
|-----------|---------|----------|-------|----------|---------|---------|----------|----------|
| rs1456315 | TT      | 20(0.30) | 39(0.51) |          | Ref     |         |          |          |
|           | TC      | 28(0.42) | 27(0.36) | 2.022    | 0.95–4.303 | 3.38    |          | 0.06603  |
|           | CC      | 17(0.26) | 10(0.13) | 3.315    | 1.283–8.563 | 6.38    |          | 0.01152* |
|           | TC+CC   | 45(0.69) | 37(0.49) | 2.372    | 1.186–4.741 | 6.08    |          | 0.01369* |
|           | T       | 68(0.52) | 105(0.69) |          | Ref     |         |          |          |
|           | C       | 62(0.48) | 47(0.31) | 2.037    | 1.252–3.31 | 8.31    |          | 0.00394* |
| rs16901946| AA      | 63(0.95) | 76(1.00) |          | Ref     |         |          |          |
|           | AG      | 3(0.05)  | 0       | 8.433    | 0.428–166.329 | 3.53    |          | 0.06030  |
|           | GG      | 0       | 0       | 1.205    | 0.024–61.576 | nan    |          | 1.00000  |
|           | AG+GG   | 3(0.05)  | 0       | 8.433    | 0.428–166.329 | 3.53    |          | 0.06030  |
|           | A       | 129(0.98)| 152(1.00) |          | Ref     |         |          |          |
|           | G       | 3(0.02)  | 0       | 8.243    | 0.422–161.067 | 3.49    |          | 3.49     |
| rs13252298| AA      | 33(0.50) | 30(0.42) |          | Ref     |         |          |          |
|           | AG      | 25(0.38) | 32(0.45) | 0.710    | 0.346–1.459 | 0.87    |          | 0.35092  |
|           | GG      | 8(0.12)  | 9(0.13)  | 0.808    | 0.276–2.363 | 0.15    |          | 0.69684  |
|           | AG+GG   | 33(0.50) | 41(0.58) | 0.732    | 0.373–1.436 | 0.83    |          | 0.36333  |
|           | A       | 91(0.69) | 92(0.65) |          | Ref     |         |          |          |
|           | G       | 41(0.31) | 50(0.35) | 0.829    | 0.501–1.373 | 0.53    |          | 0.46605  |
| rs1016343 | CC      | 51(0.78) | 53(0.70) |          | Ref     |         |          |          |
|           | CT      | 14(0.22) | 21(0.28) | 0.693    | 0.318–1.508 | 0.86    |          | 0.35393  |
|           | TT      | 0       | 1(0.02)  | 0.346    | 0.014–8.696 | 0.95    |          | 0.32882  |
|           | CT+CC   | 14(0.22) | 22(0.29) | 0.661    | 0.305–1.432 | 1.11    |          | 0.29260  |
|           | C       | 116(0.89)| 127(0.85) |          | Ref     |         |          |          |
|           | T       | 14(0.11) | 23(0.15) | 0.666    | 0.327–1.356 | 1.27    |          | 0.26070  |

* = significant after Bonferroni correction

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p = 0.003) in ≤ 57-year-old patients, even after Bonferroni correction (p = 0.012) (Table 4). No association was observed with > 57-year-old patients for all of the four tested SNPs (Table 5).

Further, genotype frequencies were analyzed based on gender and the data are shown in Tables 6 and 7. No statistically significant association was observed in males with PRNCR1 SNPs (Table 6). In contrast to this, a statistically significant risk association was observed in females with the rs1456315 SNP (Table 7). The SNP rs1456315 homozygous variant “CC” frequency was 5.6 fold higher in female patients compared to those in controls and showed a significant association in female CRC patients (OR: 5.625; χ² 2 = 8.57; CI: 1.648–19.202; p = 0.003), even after Bonferroni correction (p = 0.012). The additive alleles of rs1456315 “TC+CC” showed significant risk association in female cases compared to controls (OR: 2.25; χ² 2 = 4.77; CI: 1.081–4.683; p = 0.02). The SNP rs1456315 minor allele “C” frequency was 2.3 fold higher in female patients compared to that in the matched control (OR: 2.329; χ² 2 = 9.36; CI: 1.348–4.026; p = 0.002), even after Bonferroni correction (p = 0.008) (Table 7).

The association of PRNCR1 SNPs with CRC was also analyzed by stratifying the tumor samples based on tumor location. The patients were divided into those with tumors located in the colon region and those with tumors in the rectal region. Interestingly, rs1456315 showed...
statistically significant association in the colon while in rectal cancer no significant association was observed in CRC patients (Tables 8 and 9).

Patients with colon cancer showed significantly higher risk (2.2 fold) with SNP rs1456315 homozygous variant genotype "CC" when compared to healthy individuals (OR: 2.227; $\chi^2 = 4.64; \text{CI: } 1.067–4.647; p = 0.03$). The additive alleles "TC+CC" genotype showed significant association in CRC patients compared to healthy individuals (OR: 1.798; $\chi^2 = 4.30; \text{CI: } 1.030–3.136; p = 0.03$). The minor allele "C" frequency was also showed significantly higher association in patients when compared to healthy controls (OR: 1.619; $\chi^2 = 5.97; \text{CI: } 2.385–1.099; p = 0.01$), even after Bonferroni correction (p = 0.04) (Table 8). However, the genotype association was not observed for rectal CRC patients with any of the PRNCR1 SNPs (Table 9).

Linkage disequilibrium analysis showed that there was very low association among the analyzed SNPs in controls. The $r^2$ values are slightly different in colon cancer cases compared to controls (Fig 1). LDproxy analysis for rs1456315 showed a close association with rs13254738 ($r^2 = 0.66$). LDproxy analysis of rs16901946 showed a close association with four PRNCR1 SNPs rs12682421 ($r^2 = 1$), rs77236771 ($r^2 = 0.89$), rs139756632 ($r^2 = 0.86$) and rs75414904 ($r^2 = 0.86$). SNP rs1016343 showed close association with rs7837848 ($r^2 = 0.88$).

The RNAsnp prediction showed that rs1456315 T→C allele substitution resulted in

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**Table 5. The association between PRNCR1 gene polymorphism and the risk of colorectal cancer in patients older than 58 years.** Percentage of total cases are shown in parentheses.

| SNP       | Variant | cases | controls | OR    | CI       | $\chi^2$ Value | P-Value |
|-----------|---------|-------|----------|-------|----------|---------------|---------|
| rs1456315 | TT      | 30(0.38) | 22(0.41) | 1.013 | 0.461–2.223 | 0.00 | 0.97490 |
|           | TC      | 29(0.37) | 21(0.39) | 1.267 | 0.503–3.192 | 0.25 | 0.61586 |
|           | CC      | 19(0.25) | 11(0.20) | 1.100 | 0.541–2.235 | 0.07 | 0.79217 |
|           | TC+CC   | 48(0.62) | 32(0.59) |       |          |               |         |
|           | T       | 89(0.57) | 65(0.60) |       |          |               |         |
|           | C       | 67(0.43) | 43(0.40) | 1.138 | 0.691–1.874 | 0.26 | 0.61158 |
| rs16901946| AA      | 77(0.99) | 52(0.96) |       |          |               |         |
|           | AG      | 1(0.01)  | 2(0.04)  | 0.338 | 0.030–3.821 | 0.84 | 0.35868 |
|           | GG      | 0        | 0        | 0.677 | 0.013–34.677 | nan  | 1.00000 |
|           | AG+GG   | 1(0.01)  | 2(0.04)  | 0.338 | 0.030–3.821 | 0.84 | 0.35868 |
|           | A       | 155(0.99)| 106(0.98)|       |          |               |         |
|           | G       | 1(0.01)  | 2(0.02)  | 0.342 | 0.031–3.819 | 0.83 | 0.58225 |
| rs13252298| AA      | 41(0.53) | 28(0.54) |       |          |               |         |
|           | AG      | 34(0.44) | 19(0.37) | 1.222 | 0.584–2.559 | 0.28 | 0.59458 |
|           | GG      | 3(0.04)  | 5(0.10)  | 0.410 | 0.091–1.855 | 1.41 | 0.23563 |
|           | AG+GG   | 37(0.47) | 24(0.46) | 1.053 | 0.521–2.127 | 0.02 | 0.88590 |
|           | A       | 116(0.74)| 75(0.72) |       |          |               |         |
|           | G       | 40(0.27) | 29(0.28) | 0.892 | 0.510–1.560 | 0.16 | 0.68813 |
| rs1016343 | CC      | 49(0.64) | 39(0.72) |       |          |               |         |
|           | CT      | 23(0.30) | 13(0.24) | 1.408 | 0.633–3.133 | 0.71 | 0.40053 |
|           | TT      | 5(0.06)  | 2(0.04)  | 1.990 | 0.366–10.815 | 0.66 | 0.41819 |
|           | CT+CC   | 28(0.36) | 15(0.28) | 1.486 | 0.698–3.161 | 1.06 | 0.30296 |
|           | C       | 121(0.79)| 91(0.84) |       |          |               |         |
|           | T       | 33(0.21) | 17(0.16) | 1.460 | 0.766–2.783 | 1.33 | 0.24882 |

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a minimum free energy (MFE) of (-85.70)–(-85.10) kcal/mol. The base pair probabilities of rs14563153 wild type (T allele) and variant (C allele) were different.

**Discussion**

To the best of our knowledge, no study investigating the impact of PRNCR1 variants on colorectal cancer association in a Saudi population has been conducted to date. All of the PRNCR1 polymorphisms are located in the exon region [10]. Among the four SNPs included in our study, in overall case control analysis and stratified analysis, only the rs1456315 (C/T) SNP was associated with cancer risk. In colorectal cancer patients, there was a significant increase in the C/C genotype compared to controls. The remaining three SNPs, rs16901946 (A/G), rs13252298 (A/G), and rs1016343 (C/T) did not show any association. Stratified analysis of our study showed that the association between CRC and rs1456315 was substantial in a subgroup of females while for males it is not significant. Genetic polymorphism of rs1456315 affecting CRC varies between genders, which was consistent with a recent report by Wang et al. [11]. This suggested that gender-related differences are observed in lncRNA PRNCR1. In addition, we also found that the association between CRC and rs1456315 was significant in the female population, which indicates that the role of rs1456315 in CRC susceptibility was different in the diverse population. In stratification analysis, we observed that SNP rs1456315 was significantly associated with the tumor location of CRC. We observed that SNP rs1456315

| SNP          | Variant | patients Cases | Controls | OR   | CI         | χ² Value | P- Value |
|--------------|---------|----------------|----------|------|------------|----------|----------|
| rs1456315    | TT      | 28(0.34)       | 28(0.39) | Ref  |            |          |          |
|              | TC      | 33(0.40)       | 26(0.37) | 1.269| 0.609–2.644| 0.41     | 0.52404  |
|              | CC      | 21(0.26)       | 17(0.24) | 1.235| 0.540–2.823| 0.25     | 0.61616  |
|              | TC+CC   | 54(0.66)       | 43(0.61) | 1.256| 0.649–2.428| 0.46     | 0.49812  |
|              | T       | 89(0.54)       | 82(0.58) | Ref  |            |          |          |
|              | C       | 75(0.46)       | 60(0.42) | 1.152| 0.732–1.812| 0.37     | 0.54113  |
| rs16901946   | AA      | 79(0.95)       | 70(0.99) | Ref  |            |          |          |
|              | AG      | 4(0.05)        | 1(0.01) | 3.544| 0.387–32.464| 1.42     | 0.23387  |
|              | GG      | 0              | 0       | 0.887| 0.017–45.280| nan      | 1.00000  |
|              | AG+GG   | 4(0.05)        | 1(0.01) | 3.544| 0.387–32.464| 1.42     | 0.23387  |
|              | A       | 162(0.98)      | 141(0.99)| Ref  |            |          |          |
|              | G       | 4(0.02)        | 1(0.01) | 3.481| 0.385–31.512| 1.39     | 0.38167  |
| rs13252298   | AA      | 40(0.48)       | 35(0.51) | Ref  |            |          |          |
|              | AG      | 36(0.43)       | 26(0.38) | 1.212| 0.615–2.388| 0.31     | 0.57916  |
|              | GG      | 7(0.08)        | 8(0.12) | 0.766| 0.252–2.326| 0.22     | 0.63702  |
|              | AG+GG   | 43(0.52)       | 34(0.49) | 1.107| 0.584–2.096| 0.10     | 0.75592  |
|              | A       | 116(0.70)      | 96(0.70) | Ref  |            |          |          |
|              | G       | 50(0.30)       | 42(0.30) | 0.985| 0.603–1.610| 0.00     | 0.95264  |
| rs1016343    | CC      | 60(0.73)       | 46(0.65) | Ref  |            |          |          |
|              | CT      | 19(0.23)       | 23(0.32) | 0.633| 0.309–1.300| 1.56     | 0.21145  |
|              | TT      | 3(0.04)        | 2(0.03) | 1.150| 0.184–7.169| 0.02     | 0.88092  |
|              | CT+CC   | 22(0.27)       | 25(0.35) | 0.675| 0.338–1.345| 1.26     | 0.26237  |
|              | C       | 139(0.85)      | 115(0.81)| Ref  |            |          |          |
|              | T       | 25(0.15)       | 27(0.19) | 0.766| 0.421–1.392| 0.77     | 0.38118  |

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showed significant risk with colon cancer in a Saudi population. SNP rs1456315 was associated with an increased risk of CRC, especially for younger individuals. Previous studies reported that rs1456315 was associated with the association of colorectal cancer, gastric cancer and prostate cancer [7, 12, 13]. In our study, we observed that SNP rs1456315 is significantly associated with CRC risk; this finding is consistent with the findings of Chung et al. [14]. Chung et al. observed that PRNCR1 has a significant role in the prostate by regulating AR activity [14]. Several other studies supported that AR also participated in the pathological development of CRC through that TGF\( \beta \) pathway [15] [16].

In the present study, we observed that there is no significant association of PRNCR1 rs16901946 genotypes in colorectal cancer patients. Our results are inconsistent with Li et al. who reported that there was no association of rs16901946 with colorectal cancer [7] and gastric cancer [12]. However, recent meta-analysis performed by Chu et al. [13] reported that the rs16901946 variant of PRNCR1 was found to increase the risk of cancer significantly. However, He et al. [17] reported that the PRNCR1 rs16901946 polymorphisms are significantly associated with the increased association of prostate cancer in the Eastern China population.

### Table 7. The association between PRNCR1 gene polymorphism and the risk of colorectal cancer in female patients. Percentage of total cases are shown in parentheses.

| SNP         | Variant | patients | OR (95% CI) | \( \chi^2 \) Value | P-Value |
|-------------|---------|----------|-------------|---------------------|---------|
| rs1456315   | TT      | 22(0.36) | 33(0.56)    | Ref                 |         |
|             | TC      | 24(0.39) | 22(0.37)    | 1.636 (0.742–3.609) | 1.50    | 0.22115 |
|             | CC      | 15(0.25) | 4(0.07)     | 5.625 (1.648–19.202)| 8.57    | 0.00342* |
|             | TC+CC   | 39(0.64) | 26(0.44)    | 2.250 (1.081–4.683)| 4.77    | 0.02899* |
|             | T       | 68(0.56) | 88(0.70)    | Ref                 |         |
|             | C       | 54(0.44) | 30(0.25)    | 2.329 (1.348–4.026)| 9.36    | 0.00222* |
| rs16901946  | AA      | 61(1.00) | 58(0.98)    | Ref                 |         |
|             | AG      | 0        | 1(0.02)     | 0.317 (0.013–7.940)| 1.04    | 0.30722 |
|             | GG      | 0        | 0           | 0.951 (0.019–48.727)| nan     | 1.00000 |
|             | AG+GG   | 0        | 1(0.02)     | 0.317 (0.013–7.940)| 1.04    | 0.30722 |
|             | A       | 122(1.00)| 117(0.99)   | Ref                 |         |
|             | G       | 0        | 1(0.01)     | 0.320 (0.013–7.927)| 1.04    | 0.81875 |
| rs13252298  | AA      | 34(0.56) | 23(0.43)    | Ref                 |         |
|             | AG      | 23(0.38) | 25(0.46)    | 0.622 (0.287–1.351)| 1.45    | 0.22928 |
|             | GG      | 4(0.07)  | 6(0.11)     | 0.451 (0.114–1.777)| 1.34    | 0.24739 |
|             | AG+GG   | 27(0.44) | 31(0.57)    | 0.589 (0.281–1.234)| 1.98    | 0.15940 |
|             | A       | 91(0.75) | 71(0.66)    | Ref                 |         |
|             | G       | 31(0.25) | 37(0.34)    | 0.654 (0.370–1.155)| 2.15    | 0.14217 |
| rs1016343   | CC      | 40(0.67) | 46(0.79)    | Ref                 |         |
|             | CT      | 18(0.30) | 11(0.19)    | 1.882 (0.795–4.454)| 2.10    | 0.14733 |
|             | TT      | 2(0.03)  | 1(0.02)     | 2.300 (0.201–26.324)| 0.47    | 0.49183 |
|             | CT+CC   | 20(0.33) | 12(0.21)    | 1.917 (0.834–4.403)| 2.39    | 0.12247 |
|             | C       | 98(0.82) | 103(0.89)   | Ref                 |         |
|             | T       | 22(0.18) | 13(0.11)    | 1.779 (0.849–3.725)| 2.37    | 0.12356 |

* = not significant after Bonferroni correction,
* = significant after correction

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A meta-analysis study by Huang et al. [18] reported that the rs16901946 G/A polymorphism was associated with an increased overall association to cancer. A meta-analysis study showed among the PRNCR1 rs13252298 genotypes, the G/A homozygous variant is associated with cancer [18]. Li et al. reported that the rs13252298 of PRNCR1 is associated with significantly less risk of CRC [7]. A recent study in Iran reported that the PRNCR1 rs13252298 polymorphisms are significantly associated with the increased association of PCa in an Iranian population [19]. This is in contrast with our present study. There is no significant association with the SNP rs13252298 for the Saudi population.

Huang et al. reported that the SNP of PRNCR1 rs1016343 T/C polymorphism was associated with an increased overall incidence of cancer [18]. Chu et al. reported that the rs1016343 increased the risk of cancer in the dominant model and additive model with a 24% increased risk of cancer [13]. In the meta-analysis study, they found that PRNCR1 rs1016343 and rs16901946 polymorphisms were contributing to cancer risk [10], while for the Saudi population in this study there is no significant association of rs1016343 with colorectal cancer.

Recent studies demonstrated that PRNCR1 plays a vital role in cancer progression. One of the studies by Yang et al. [20] described that PRNCR1 affects and alters the androgen receptor mechanism and thus increases the risk of prostate cancer development. PRNCR1 binds to the

Table 8. Genotype frequencies of PRNCR1 gene polymorphism in colorectal tumors located in the colon area. Percentage of total cases are shown in parentheses.

| SNP      | Variant | patients | Cases | Controls | OR      | CI     | χ² Value | P- Value |
|----------|---------|----------|-------|----------|---------|--------|----------|----------|
| rs1456315 TT | 30(0.33) | 61(0.47) |       |          | 1.610   | 0.875–2.963 | 2.35   | 0.12510 |
| TC       | 38(0.42) | 48(0.37) |       |          | 1.610   | 0.875–2.963 | 2.35   | 0.12510 |
| CC       | 23(0.25) | 21(0.16) |       |          | 2.227   | 1.067–4.647 | 4.64   | 0.03131 |
| TC+CC    | 61(0.67) | 69(0.53) |       |          | 1.798   | 1.030–3.136 | 4.30   | 0.03801 |
| T        | 98(0.54) | 170(0.65) |       |          | Ref     |        | 5.97     | 0.01454 |
| C        | 84(0.46) | 90(0.35) |       |          | 1.619   | 1.099–2.385 | 5.97   | 0.01454 |
| rs16901946 AA | 90(0.99) | 128(0.98) |       |          | Ref     |        | 1.0     | 0.30090 |
| AG       | 1(0.01)  | 2(0.02)  |       |          | 0.711   | 0.064–7.962 | 0.08   | 0.51561 |
| GG       | 0        | 0        |       |          | 1.420   | 0.028–72.220 | 0.001  | 1.00000 |
| AG+GG    | 1(0.01)  | 2(0.02)  |       |          | 0.711   | 0.064–7.962 | 0.08   | 0.51561 |
| A        | 181(0.99)| 258(0.99) |       |          | Ref     |        | 1.0     | 0.30090 |
| G        | 1(0.01)  | 2(0.01)  |       |          | 0.713   | 0.064–7.919 | 0.08   | 1.09652 |
| rs13252298 AA | 47(0.52) | 58(0.47) |       |          | Ref     |        | 1.0     | 0.30090 |
| AG       | 38(0.42) | 51(0.41) |       |          | 0.919   | 0.520–1.625 | 0.08   | 0.77266 |
| GG       | 6(0.07)  | 14(0.11) |       |          | 0.529   | 0.189–1.483 | 1.50   | 0.22082 |
| AG+GG    | 44(0.48) | 65(0.53) |       |          | 0.835   | 0.486–1.437 | 0.42   | 0.51561 |
| A        | 132(0.73)| 167(0.68) |       |          | Ref     |        | 1.0     | 0.30090 |
| G        | 50(0.27) | 79(0.32) |       |          | 0.801   | 0.525–1.220 | 1.07   | 0.30090 |
| rs1016343 CC | 65(0.70) | 92(0.71) |       |          | Ref     |        | 1.0     | 0.30090 |
| CT       | 33(0.26) | 34(0.26) |       |          | 0.988   | 0.532–1.834 | 0.00   | 0.96914 |
| TT       | 4(0.04)  | 3(0.02)  |       |          | 1.947   | 0.421–9.000 | 0.75   | 0.38597 |
| CT+CC    | 27(0.30) | 37(0.29) |       |          | 1.066   | 0.590–1.924 | 0.04   | 0.83290 |
| C        | 149(0.83)| 218(0.84) |       |          | Ref     |        | 1.0     | 0.30090 |
| T        | 31(0.17) | 40(0.16) |       |          | 1.134   | 0.679–1.894 | 0.23   | 0.63116 |

*ns = not significant after Bonferroni correction,
* = significant after correction

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androgen receptor acetylated region and its relationship with DOT1L seems to be mandatory for recruitment of PCGEM to the DOT1L-mediated methylation of AR at the N-terminus [20]. Li et al. [7] found that a critical region of 8q24 might indicate predisposition to CRC. Yang et al. [3] also specified that overexpression of PRNCR1 was evidently interconnected with tumor stage and size. Furthermore, it has been found that patients with an rs1456315G polymorphism have a tumor of much larger size. It may be due to the effect of the polymorphisms on the secondary structure of PRNCR1 mRNA and thus changing its stability. Further, they also reported that the silencing of PRNCR1 inhibited the cell cycle at the G0/G1 stage demonstrating that PRNCR1 stimulates the propagation of colon cancer from epithelial cells, and ultimately might increase the tumor size in patients. Li et al. [7] reported that 5 SNPs in PRNCR1: rs1456315G, rs7007694C, rs13252298, rs1456315, and rs16901946G, showed a significant association with colorectal cancer progression. Furthermore, an increasing number of GWAS studies reported a strong association of a CpG site at Chr8: 128167809 in PRNCR1 and CRC predisposition. The PRNCR1 SNP rs1456315G and this CpG site have been reported to have strong association with each other.

The PRNCR1 catalytic subunit has been widely linked to cancer development. PRNCR1 was reported to be frequently mutated in many types of cancers, such as esophageal cancer, colorectal cancer, and breast cancer. Recently, PRNCR1 genomic variants were identified to be associated with increased cancer risk. Park et al. reported that the PRNCR1 rs1456315G and

| SNP        | Variant | patients | Cases | Controls | OR       | CI       | $\chi^2$ Value | P- Value |
|------------|---------|----------|-------|----------|----------|----------|----------------|----------|
| rs1456315  | TT      | 20(0.38) | 61(0.47) | Ref      |          |          | 0.580–2.513    | 0.25     | 0.61424 |
|            | TC      | 19(0.37) | 48(0.37) | 1.207    | 0.580–2.513 | 0.25     | 0.61424 |
|            | CC      | 13(0.25) | 21(0.16) | 1.888    | 0.802–4.446 | 2.15     | 0.14285 |
|            | TC+CC   | 32(0.62) | 69(0.53) | 1.414    | 0.734–2.727 | 1.08     | 0.29943 |
|            | T       | 59(0.57) | 170(0.65) | Ref      |          |          |                |          |
|            | C       | 45(0.43) | 90(0.35) | 1.441    | 0.905–2.292 | 2.38     | 0.12256 |
| rs16901946 | AA      | 50(0.94) | 128(0.98) | Ref      |          |          |                |          |
|            | AG      | 3(0.06)  | 2(0.02)  | 3.840    | 0.623–23.672 | 2.41     | 0.12079 |
|            | GG      | 0        | 0        | 2.545    | 0.050–129.979 | 0.001   | 1.00000 |
|            | AG+GG   | 3(0.06)  | 2(0.02)  | 3.840    | 0.623–23.672 | 2.41     | 0.12079 |
|            | A       | 103(0.97) | 258(0.99) | Ref      |          |          |                |          |
|            | G       | 3(0.03)  | 2(0.01)  | 3.757    | 0.619–22.815 | 2.37     | 0.25226 |
| rs13252298 | AA      | 27(0.51) | 58(0.47) | Ref      |          |          |                |          |
|            | AG      | 21(0.40) | 51(0.41) | 0.885    | 0.447–1.752 | 0.12     | 0.72478 |
|            | GG      | 5(0.09)  | 14(0.11) | 0.767    | 0.251–2.348 | 0.22     | 0.64176 |
|            | AG+GG   | 26(0.49) | 65(0.53) | 0.859    | 0.451–1.637 | 0.21     | 0.64447 |
|            | A       | 75(0.71) | 167(0.68) | Ref      |          |          |                |          |
|            | G       | 31(0.29) | 79(0.32) | 0.874    | 0.532–1.436 | 0.28     | 0.59427 |
| rs1016343  | CC      | 37(0.71) | 92(0.71) | Ref      |          |          |                |          |
|            | CT      | 14(0.27) | 34(0.26) | 1.024    | 0.493–2.125 | 0.00     | 0.94955 |
|            | TT      | 1(0.02)  | 3(0.02)  | 0.829    | 0.084–8.227 | 0.03     | 0.87245 |
|            | CT+CC   | 15(0.29) | 37(0.29) | 1.008    | 0.495–2.052 | 0.00     | 0.98240 |
|            | C       | 88(0.85) | 218(0.84) | Ref      |          |          |                |          |
|            | T       | 16(0.15) | 40(0.16) | 0.991    | 0.528–1.861 | 0.00     | 0.97735 |

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rs6983267 SNPs were significantly associated with melanoma patient survival. Wang et al. reported that PIK3CA rs2699887 showed notable associations with the survival of endometrial cancer patients. However, the association between rs6983267 and colorectal cancer risk as well as patient survival remains elusive.

The RNAsnp prediction showed that rs1456315 changed the RNA secondary structure of PRNCR1 (Fig 2). Therefore, we speculate that rs1456315 could be a regulatory SNP, which regulates the expression of PRNCR1 and contributes to the genetic susceptibility of colorectal cancer. The RNAsnp prediction for rs16901946 showed that there is no change in the RNA structures of PRNCR1, while the base pair probabilities of rs16901946 are slightly different. The RNAsnp prediction showed that rs13252298 and rs1016343 did not changed in the folding structures of PRNCR1, and also the base pair probabilities are the same.

The present study has some strengths and limitations. Mainly, the genetic association analysis was performed in a small cohort, and these results must be validated in larger cohorts and other populations. We enrolled native Saudi colorectal cancer patients and controls in the present study. This is the first study to report PRNCR1 variant association with cancer in a Saudi population. In conclusion, in the present study we confirmed the association of rs1456315 with colorectal cancer risk in a Saudi population.
Supporting information

S1 Table. Control data.
(PDF)

S2 Table. Cases data.
(PDF)

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