NOTCH Single Nucleotide Polymorphisms in the Predisposition of Breast and Colorectal Cancers in Saudi Patients

Ibrahim O. Alanazi, Jilani Purusottapattam Shaik, Narasimha Reddy Parine, Abdulrahman Al Naeem, Nahla A. Azzam, Majid A. Almadi, Abdulrahman M. Aljebreen, Othman Alharbi, Mohammad Saud Alanazi and Zahid Khan

Breast cancer (BC) is a heterogeneous disease and is one of the most common malignancies affecting women worldwide while colorectal cancer (CRC) is estimated to be the third common cancer and second leading cause of cancer related death globally. Both BC and CRC involve multiple genetic and epigenetic alterations in genes belonging to various signaling pathways including NOTCH that has been implicated in the development of these cancers. We investigated four single nucleotide polymorphisms, each in genes encoding NOTCH1-4 receptors for their role in susceptibility to breast and colorectal cancers in Saudi population. In this case-control study, TaqMan genotypic analysis of rs3124591 in NOTCH1 and rs3820041 in NOTCH4 did not exhibit association with breast as well as colorectal cancers. However, a strong association of rs11249433 which is in close proximity to NOTCH2 was observed with breast cancer susceptibility especially with those having an early onset of the disease. Interestingly, the rs1043994 located in NOTCH3 showed gender preference and was found to be significantly associated with colorectal cancers in males. Validation of these findings in bigger populations of different ethnicities may prove beneficial in identifying rs11249433 and rs1043994 as genetic screening markers for early detection of breast and colorectal carcinomas, respectively.

Keywords: Notch, single nucleotide polymorphism, breast cancer, colorectal cancer, genetic screening marker

INTRODUCTION

Breast cancer (BC) is a heterogeneous and one of the common disease affecting women worldwide. BC accounts for 11.7 percent (2,261 million) of all new cancer cases and 6.9 percent (684,996) of all cancer deaths globally [1]. It is the most frequently diagnosed cancer in women living in Gulf Cooperation Council countries, including Saudi Arabia [2]. In Saudi Arabia, BC is ranked first among females with an estimated number of new cases to be 3,954 (29.0%) of the 13,632 total cancer cases in women, while BC related mortality was reported to be 1,095 (20.4%) of 5,376 cancer related...
Effects are ongoing to identify reliable biomarkers for predicting human malignant neoplastic diseases. Genetic variation analysis has the potential to be utilized for screening and identifying novel prognosis genes. Single-nucleotide polymorphisms (SNPs) is one of the most common type of genetic variation that may serve as potential and distinctive genetic markers. In the present study, based on the associations with cancers from previous literature, we examined the influence of four SNPs, rs3124591, rs1124943, rs1043994, and rs3830041 which are located within or in close proximity of NOTCH1, NOTCH2, NOTCH3 and NOTCH4 genes, respectively on BC and CRC susceptibility by comparing the genotypic distribution of these SNPs in cancer cases to that of healthy subjects from Saudi Arabia.

MATERIALS AND METHODS

Study Population

The study subjects included in this case-control study comprised of women with pathologically confirmed breast cancer and age-matched female controls of Saudi Arabian ethnicity without any history of cancer. The median age at the time of breast cancer diagnosis was 53 years. The number of cases and controls examined for each SNP were as follows: NOTCH1 rs3124591 (cases n = 185; controls n = 180), NOTCH2 related rs11249433 (cases n = 113), NOTCH3 rs1043994 (cases n = 128; control n = 128), NOTCH4 rs3830041 (cases n = 120; controls n = 134). Pretreatment blood samples from breast cancer patients were collected at King Fahad Medical City, Riyadh. The number of CRC cases and controls included in this study for each SNP were as follows: NOTCH1 rs3124591 (cases n = 90; controls n = 70), NOTCH2 related rs11249433 (cases n = 185; controls n = 133), NOTCH3 rs1043994 (cases n = 182; control n = 128), NOTCH4 rs3830041 (cases n = 168; controls n = 134). Pretreatment blood samples from breast cancer patients were collected at King Fahad Medical City, Riyadh. DNA Extraction

Ethylendiaminetetraacetic acid (EDTA) containing vacutainers were used to collect approximately 3 ml of blood samples from each study participants. Genomic DNA isolation was performed utilizing QIAmp DNA blood mini kit (Catalog no. 51104, Applied Biosystems, Foster City, CA, United States) as per the manufacturer’s instructions. Spectrophotometric quantitation and purity of the extracted DNA was done on NanoDrop 8000 (Thermo Scientific, Waltham, MA, United States).

SNP Selection and Genotyping

A total of four single nucleotide polymorphism in NOTCH1, NOTCH2, NOTCH3 and NOTCH4 receptor genes were selected from previous literature [18–24]. TaqMan allelic discrimination assays were used to genotype the SNPs based on Livak’s method [25]. Briefly, for each sample, 20 ng of purified genomic DNA was mixed with 5.0 µl of 2× TaqMan genotyping Master Mix (Catalog no. 4371355, Applied Biosystems, Foster City, CA, United States) and 0.25 µl of 40× TaqMan SNP genotyping assay (Catalog no. 4351379, Assay ID: C____189,059_10; C__31617470_30; C____7494157_10; C__27523194_10, Thermo Fisher Scientific, United States) containing the primers and probe in a total volume of 10 µl performed in Fast Optical 96-Well Reaction Plate (Catalog no. 4346906, Applied Biosystems, Foster City, CA,
United States). The genotypes were determined by endpoint reading on QuantStudio 7 Flex Real Time PCR system (Applied Biosystems, Foster City, CA, United States). The instrument was programmed as follows: pre-read at 60°C for 30 s, polymerase activation at 95°C for 10 min, 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min followed by post-read at 60 °C for 30 s. TaqMan Genotyper Software version 1.4 was used to automatically analyze the data and make the genotype calls.

Statistical Analysis

Frequencies for the three genotypes and alleles for each SNP were computed and tests for deviation from Hardy-Weinberg equilibrium and tests for association were performed using publicly available web-based tool at https://ihg.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl. Genetic association of each SNP with breast and colorectal cancer were determined by case-control comparisons using the chi-square test and odds ratios (OR), and 95% CI. A $p$-value of $\leq 0.05$ was considered as significant. Additionally, since we analyzed 4 SNPs in total, Bonferroni’s correction for multiple comparison was applied with an $\alpha = 0.0125$ considered as significant.

RESULTS

We examined the genotypes of four germline SNPs residing in NOTCH1 - rs3124591, NOTCH2 - rs11249433, NOTCH3 - rs1043994, and NOTCH4 - rs3830041 to determine their association with breast and colorectal cancer risk in Saudi Arabian patients. The rs3124591 is a 3 Prime UTR variant in NOTCH1, rs11249433 resides in a linkage disequilibrium block neighboring NOTCH2, rs1043994 is a synonymous variant coding for alanine at protein position 202 of NOTCH3 and rs3830041 is an intronic variant in NOTCH4 receptor.

Association of Genetic Variants in NOTCH Receptors With Breast and Colorectal Cancers

Breast Cancer

The distribution of genotypes for all the four SNPs in control group as well as NOTCH3 rs1043994 and NOTCH4 rs3830041 in breast cancers followed Hardy-Weinberg equilibrium while SNPs rs3124591 in NOTCH1 ($p = 0.046275$) and NOTCH2 related rs11249433 ($p = 0.000095$) in breast cancers deviated from Hardy-Weinberg equilibrium (Table 1). Clinicopathological and demographic data along with genotypes of the examined SNPs for each breast cancer cases and controls are presented in Supplementary Table S1.

The distributions of genotype and allele frequencies of the examined SNPs for each breast cancer cases and controls are presented in Supplementary Table S1. In the overall analysis, only rs11249433 SNP that is in linkage disequilibrium block neighboring NOTCH2, rs1043994 is a synonymous variant coding for alanine at protein position 202 of NOTCH3 and rs3830041 is an intronic variant in NOTCH4 receptor.

Table 1 | Test for deviation from Hardy-Weinberg equilibrium.

| SNP ID   | Genotype | Controls $n$ (frequency) | HWE $p$-value | Cancer $n$ (frequency) | HWE $p$-value |
|----------|----------|--------------------------|---------------|------------------------|---------------|
|          |          |                           |               |                        |               |
| rs3124591| CC       | 52 (0.74)                | 0.217048      | 142 (0.75)             | 0.046275      |
|          | CT       | 18 (0.26)                |               | 48 (0.25)              |               |
|          | TT       | 00 (0.00)                |               | 00 (0.00)              |               |
| rs11249433| GG      | 19 (0.14)                | 0.730739      | 14 (0.08)              | 0.000095      |
|          | GA       | 65 (0.49)                |               | 112 (0.60)             |               |
|          | AA       | 49 (0.37)                |               | 59 (0.32)              |               |
| rs1043994| GG       | 64 (0.5)                 | 0.053619      | 103 (0.568)            | 0.507910      |
|          | GA       | 59 (0.48)                |               | 70 (0.385)             |               |
|          | AA       | 05 (0.04)                |               | 09 (0.049)             |               |
| rs3830041| GC       | 67 (0.42)                | 0.958591      | 91 (0.49)              | 0.445045      |
|          | CT       | 61 (0.46)                |               | 75 (0.40)              |               |
|          | TT       | 16 (0.12)                |               | 20 (0.11)              |               |

HWE, Hardy-Weinberg equilibrium; $p \leq 0.05$ was considered significant and are depicted in bold and deviated from HWE.
### TABLE 2 | NOTCH receptors SNPs genotype and allele frequencies in breast cancer cases and control population.

| SNP ID             | Genotype | Controls n (frequency) | Breast cancer n (frequency) | OR (95% CI)       | \( \chi^2 \) -value | p-value* |
|--------------------|----------|------------------------|----------------------------|-------------------|---------------------|----------|
| rs3124591, NOTCH1  | CC       | 52 (0.74)              | 142 (0.75)                 | Ref               |                      |          |
|                    | CT       | 18 (0.26)              | 48 (0.25)                  | 0.977 (0.521–1.830) | 0.01                | 0.94090  |
|                    | TT       | 00 (0.00)              | 00 (0.00)                  | 0.366 (0.007–18.806) | na                  | 1.00000  |
|                    | Allele   |                       |                            |                   |                     |          |
|                    | C        | 122 (0.87)             | 332 (0.87)                 | Ref               | 0.977 (0.521–1.830) | 0.01    |
|                    | T        | 18 (0.13)              | 48 (0.13)                  | 0.980 (0.549–1.750) | 0.00                | 0.94536  |
|                    | Allele   |                       |                            |                   |                     |          |
| rs11249433, NOTCH2 | GG       | 19 (0.14)              | 14 (0.08)                  | Ref               | 0.977 (0.521–1.830) | 0.01    |
|                    | GA       | 65 (0.49)              | 112 (0.60)                 | 2.338 (1.099–4.975) | 5.04                | 0.02478  |
|                    | AA       | 49 (0.37)              | 59 (0.32)                  | 1.634 (0.744–3.591) | 1.51                | 0.21943  |
|                    | Allele   |                       |                            |                   |                     |          |
| rs1043994, NOTCH3  | GG       | 19 (0.14)              | 14 (0.08)                  | Ref               | 0.977 (0.521–1.830) | 0.01    |
|                    | GA       | 65 (0.49)              | 112 (0.60)                 | 2.338 (1.099–4.975) | 5.04                | 0.02478  |
|                    | AA       | 49 (0.37)              | 59 (0.32)                  | 1.634 (0.744–3.591) | 1.51                | 0.21943  |
|                    | Allele   |                       |                            |                   |                     |          |
| rs3830041, NOTCH4  | GG       | 19 (0.14)              | 14 (0.08)                  | Ref               | 0.977 (0.521–1.830) | 0.01    |
|                    | GA       | 65 (0.49)              | 112 (0.60)                 | 2.338 (1.099–4.975) | 5.04                | 0.02478  |
|                    | AA       | 49 (0.37)              | 59 (0.32)                  | 1.634 (0.744–3.591) | 1.51                | 0.21943  |

OR 95% CI, Odds Ratio and 95% Confidence Interval; na, not analyzable.
* p ≤ 0.05 was considered significant and are depicted in bold.

### TABLE 3 | NOTCH receptors SNPs genotype and allele frequencies in colorectal cancer cases and control population.

| SNP ID             | Genotype | Controls n (frequency) | CRC n (frequency) | OR (95% CI)       | \( \chi^2 \) -value | p-value* |
|--------------------|----------|------------------------|-------------------|-------------------|---------------------|----------|
| rs3124591, NOTCH1  | CC       | 82 (0.80)              | 74 (0.77)         | Ref               |                      |          |
|                    | CT       | 21 (0.20)              | 22 (0.23)         | 1.161 (0.591–2.281) | 0.19                | 0.66499  |
|                    | TT       | 00 (0.00)              | 00 (0.00)         | 1.107 (0.022–56.510) | na                  | 1.00000  |
|                    | Allele   |                       |                   |                   |                     |          |
|                    | C        | 185 (0.90)             | 170 (0.89)        | Ref               | 1.161 (0.591–2.281) | 0.19    |
|                    | T        | 21 (0.10)              | 22 (0.11)         | 1.140 (0.605–2.147) | 0.16                | 0.68477  |
| rs11249433, NOTCH2 | GG       | 57 (0.42)              | 91 (0.49)         | Ref               | 1.161 (0.591–2.281) | 0.19    |
|                    | GA       | 70 (0.50)              | 72 (0.51)         | 0.836 (0.375–1.864) | 0.39                | 0.53077  |
|                    | AA       | 56 (0.40)              | 53 (0.38)         | 0.769 (0.338–1.750) | 0.39                | 0.53077  |
|                    | Allele   |                       |                   |                   |                     |          |
| rs1043994, NOTCH3  | GG       | 84 (0.60)              | 76 (0.57)         | Ref               | 0.836 (0.375–1.864) | 0.39    |
|                    | GA       | 52 (0.37)              | 56 (0.42)         | 1.190 (0.730–1.940) | 0.49                | 0.48457  |
|                    | AA       | 03 (0.02)              | 2 (0.01)          | 0.737 (0.120–4.529) | 0.11                | 0.74081  |
|                    | Allele   |                       |                   |                   |                     |          |
| rs3830041, NOTCH4  | GG       | 220 (0.79)             | 208 (0.78)        | Ref               | 1.190 (0.730–1.940) | 0.49    |
|                    | GA       | 182 (0.65)             | 178 (0.63)        | 0.903 (0.639–1.276) | 0.34                | 0.56221  |
|                    | AA       | 84 (0.60)              | 76 (0.57)         | Ref               | 0.903 (0.639–1.276) | 0.34    |
|                    | Allele   |                       |                   |                   |                     |          |

CRC, colorectal cancer; OR 95% CI, odds ratio and 95% Confidence Interval; na, not analyzable.
* p ≤ 0.05 was considered significant and are depicted in bold.
to 49% in the control group. Women that harbor heterozygous GA genotype for this SNP were at 2.3-fold higher risk of developing carcinoma of the breast compared to those having GG genotype (OR = 2.338, χ² = 5.04, p = 0.02478). The other three SNPs, rs3124591 (NOTCH1), rs1043994 (NOTCH3), and rs3830041 (NOTCH4) were not significantly associated with the predisposition of breast carcinoma in the overall analysis (Table 2).

Colorectal Cancer
Pathologically confirmed colorectal cancer cases and age as well as gender-matched controls from Saudi Arabian population
without prior history of cancer were examined to find an association of genetic variants in NOTCH receptors and susceptibility of CRC. The genotypes distributions of all the SNPs were in accordance with Hardy-Weinberg equilibrium in control population. In CRC patients, genotypes of all SNPs except NOTCH3 rs1043994 followed Hardy-Weinberg equilibrium (Table 1). The clinicopathological and demographic data for each colorectal cancers and controls along with genotypes of analyzed SNPs are presented in Supplementary Table S2.
The genotypes and allele frequencies in CRCs and controls are shown in Table 3. The genotypic and allelic frequencies in CRC patients and control group were not significantly different and hence these SNPs in the NOTCH receptors were not associated with the susceptibility of colorectal cancers in our cohort in the overall analysis (Table 3).

| SNP ID          | Genotype | Controls n (frequency) | CRC n (frequency) | OR (95% CI)      | \( \chi^2 \)-value | p-value* |
|-----------------|----------|------------------------|-------------------|------------------|-------------------|----------|
| rs3124591, NOTCH1 | CC       | 45 (0.9)               | 39 (0.76)         | Ref              | 2.769 (0.896–8.555) | 3.30     | 0.06922  |
|                 | CT       | 05 (0.1)               | 12 (0.24)         |                  | 1.152 (0.022–59.409) | na       | 1.00000  |
|                 | TT       | 00 (0)                 | 0 (0)             | Ref              |                  |          |          |
| Allele          | C        | 95 (0.95)              | 90 (0.88)         | Ref              |                  |          |          |
|                 | T        | 05 (0.05)              | 12 (0.12)         |                  |                  |          |          |
|                 | G        | 08 (0.11)              | 11 (0.13)         | Ref              |                  |          |          |
|                 | A        | 35 (0.48)              | 41 (0.49)         |                  |                  |          |          |
|                 | AA       | 30 (0.41)              | 31 (0.37)         |                  |                  |          |          |
| Allele          | G        | 51 (0.35)              | 63 (0.38)         | Ref              |                  |          |          |
|                 | A        | 95 (0.65)              | 103 (0.62)        |                  |                  |          |          |
| rs11249433, NOTCH2 | GG       | 49 (0.67)              | 40 (0.51)         | Ref              |                  |          |          |
|                 | GA       | 23 (0.32)              | 37 (0.47)         |                  | 1.971 (1.011–3.841) | 4.01     | 0.04514  |
|                 | AA       | 01 (0.01)              | 1 (0.01)          |                  | 1.225 (0.074–20.207) | 0.02     | 0.88698  |
| Allele          | G        | 121 (0.83)             | 117 (0.75)        | Ref              |                  |          |          |
|                 | A        | 25 (0.17)              | 39 (0.25)         |                  |                  |          |          |
| rs1043994, NOTCH3 | GG       | 49 (0.67)              | 40 (0.51)         | Ref              |                  |          |          |
|                 | GA       | 23 (0.32)              | 37 (0.47)         |                  |                  |          |          |
|                 | AA       | 01 (0.01)              | 1 (0.01)          |                  |                  |          |          |
| Allele          | G        | 121 (0.83)             | 117 (0.75)        | Ref              |                  |          |          |
|                 | A        | 25 (0.17)              | 39 (0.25)         |                  |                  |          |          |
| rs3830041, NOTCH4 | CC       | 39 (0.53)              | 40 (0.54)         | Ref              |                  |          |          |
|                 | CT       | 28 (0.38)              | 34 (0.41)         |                  |                  |          |          |
|                 | TT       | 6 (0.08)               | 4 (0.05)          |                  |                  |          |          |
| Allele          | G        | 51 (0.35)              | 63 (0.38)         | Ref              |                  |          |          |
|                 | A        | 95 (0.65)              | 103 (0.62)        |                  |                  |          |          |
| rs11249433, NOTCH2 | GG       | 49 (0.67)              | 40 (0.51)         | Ref              |                  |          |          |
|                 | GA       | 23 (0.32)              | 37 (0.47)         |                  |                  |          |          |
|                 | AA       | 01 (0.01)              | 1 (0.01)          |                  |                  |          |          |
| Allele          | G        | 121 (0.83)             | 117 (0.75)        | Ref              |                  |          |          |
|                 | A        | 25 (0.17)              | 39 (0.25)         |                  |                  |          |          |
| rs1043994, NOTCH3 | GG       | 49 (0.67)              | 40 (0.51)         | Ref              |                  |          |          |
|                 | GA       | 23 (0.32)              | 37 (0.47)         |                  |                  |          |          |
|                 | AA       | 01 (0.01)              | 1 (0.01)          |                  |                  |          |          |
| Allele          | G        | 121 (0.83)             | 117 (0.75)        | Ref              |                  |          |          |
|                 | A        | 25 (0.17)              | 39 (0.25)         |                  |                  |          |          |

CRC, colorectal cancer; OR 95% CI, odds ratio and 95% Confidence Interval; na, not analyzable.

* p ≤ 0.05 was considered significant and are depicted in bold.
Genetic Variants in NOTCH Receptors and Age of Onset of Breast and Colorectal Cancers

Breast Cancer

To determine the association between genetic variants in NOTCH receptors and age of onset of breast cancer, we segregated the cases and control groups based on the median age of breast cancer diagnosis as ≤53 years and >53 years. As observed for the overall analysis, the rs11249433 (NOTCH2) SNP was found to be significantly associated with early onset (≤53 years of age at diagnosis) of breast cancer. The GA genotype of NOTCH2 related rs11249433 SNP conferred about 4.7 fold higher risk of developing carcinoma of the breast before or till the age of 53 years relative to those having GG genotype (OR = 4.692, \( \chi^2 = 7.26, p = 0.00707 \)) (Table 4). This association was maintained even after Bonferroni’s correction for multiple comparisons. The other variants, rs3124591 (NOTCH1), rs1043994 (NOTCH3), and rs3830041 (NOTCH4) did not influence the early onset of breast malignancies in our patients. Similarly, the distribution of genotype and allele frequencies of all the four SNPs were comparable in controls and breast cancer patients whose age at the time of diagnosis was >53 years and hence were not associated with the late onset of the disease (Table 4).

Colorectal Cancer

Colorectal cancer cases and controls were segregated according to the median age at the time of disease diagnosis as ≤58 years and >58 years. None of the four SNPs examined in the NOTCH receptors were found to be significantly associated with the age of onset of colorectal cancers (Table 5).

Association of SNPs in NOTCH Receptors With Colorectal Cancer Based on Gender

In order to evaluate whether gender played any role in the association of NOTCH1-rs3124591, NOTCH2-rs11249433, NOTCH3-rs1043994, and NOTCH4-rs3830041 with colorectal cancers, the study subjects were grouped as males and females for counting the genotype and allele frequencies. The distributions of these frequencies are depicted in Table 6. The rs1043994 SNP in the NOTCH3 receptor showed statistically significant association with colorectal cancers in males. The GA heterozygous males were about 2-fold higher risk of developing CRC relative to those with homozygous GG genotype (OR = 1.971, \( \chi^2 = 4.01, p = 0.04514 \)) (Table 6). The other three SNPs were not associated with CRCs in males. Moreover, we did not observe any of the four SNPs to be associated with CRCs in females in our population.

DISCUSSION

Notch signaling pathway is highly conserved molecular cell signaling pathway which plays important roles in proliferation, differentiation, cell fate specification, homeostasis and angiogenesis. Additionally, notch signaling is considered as one of the most common pathway implicated in cancer metastasis [26]. Several investigations have led to the conclusion that alterations in notch signaling pathway are associated with the development of various cancers including colon [15] and breast [27]. Most of the diseases including cancer are result of the interaction between genetic and environmental factors. A number of studies have indicated that genetic variation contributes in part toward the susceptibility of common diseases such as diabetes and cancer [28–30]. The identification of genetic variation associated with cancer may assist in revealing the underlying pathophysiological processes in the initiation and progression of the disease. There has been an increased interest in the most common functional germline polymorphisms on clinical outcomes for patients with cancer. The presence of genetic variation in the human genome can be found in different forms and frequencies throughout the genome. Among these forms are single nucleotide polymorphisms which are considered as the main source of genetic variation in human genome and account for about 90 percent of all human genetic variations. They occur roughly every 100–300 bases [31].

Several studies have shown that SNPs in NOTCH receptors are linked to risk and prognosis of a number of diseases. For examples, SNPs in NOTCH1 and NOTCH2 are associated with risk of breast carcinoma [19, 22]. Genetic variants in NOTCH3 gene have been shown to be associated with cerebral small vessel disease [32], while NOTCH4 variants linked to Alzheimer’s disease [33]. In the present study, we evaluated for the first time the association of NOTCH1, rs3124591; NOTCH2, rs11249433; NOTCH3, rs1043994, and NOTCH4, rs3830041 SNPs with the susceptibility of breast and colorectal cancers in Saudi population.

In the overall analysis, except for rs11249433 which is in close proximity to NOTCH2 gene that showed significant association with breast cancer, none of the other SNPs were found to confer increased risk either in breast or colorectal cancers in our population. Investigations on the association of NOTCH1 rs3124591, NOTCH3 rs1043994, and NOTCH4 rs3830041 with the risk of human cancers are rare, however, several studies have shown a link between NOTCH2 related rs11249433 and increased risk of breast cancer especially in women of European ancestry [18–21]. The rs11249433 variant is located in the pericentromeric region at 1p11.2 and NOTCH2, a transmembrane coding gene and FCGR1B (low-affinity Fc gamma receptor family) are the nearest genes to this SNP. Hunter’s group conducted a large genome-wide scan plus two stages of follow-up in 10,263 controls and 9,335 cases and found conclusive statistically significant association of NOTCH2 related rs11249433 with breast cancer [18]. They further investigated 6,386 cases for which estrogen receptor (ER) status was available to reveal that this association was more apparent for ER+ relative to ER-breast tumor. Similar association of NOTCH2-rs11249433 with ER status was not found in our breast cancer cohort.
Since cancer-associated SNPs have been shown to be linked to alterations in gene expression, Prokunina-Olsson and colleagues examined and reported that the risk genotypes of rs11249433 have a positive association with NOTCH2 mRNA expression in TP53 wild-type/ER+ breast cancers [19]. Campa et al confirmed the association of NOTCH2 rs11249433 with breast cancer risk but did not find statistically significant interaction with nine established risk factors such as age at menarche, parity, age at menopause, use of hormone replacement therapy, family history, height, body mass index, smoking status, and alcohol consumption [20]. Furthermore, a comprehensive meta-analysis comprising 90,154 cases and 137,238 controls was conducted by Wu et al to assess the relationship between the NOTCH2 rs11249433 polymorphism and breast cancer susceptibility. Their analysis showed that rs11249433 polymorphism poses significant risk in Caucasians but not in Africans and East Asians [21]. The lack of significant association between NOTCH2 rs11249433 and breast cancer risk in Chinese population as well shown by Jiang et al suggest ethnic specificity for this locus in conferring disease susceptibility [34]. Our finding of a significant association of NOTCH2 rs11249433 with the risk of breast cancer suggests that Saudi population may be closer to the Europeans than to Africans or Asians in terms of genetic susceptibility to breast cancer. Moreover, it has been demonstrated that the NOTCH2 rs11249433 exhibited a stronger association with the development of breast cancer especially with ER-positive tumors compared to ER-negative tumors [18, 35]. However, Campa et al did not observe similar association of this SNP with risk of breast cancer by ER status [20]. Another study indicated that the NOTCH2 rs11249433 was associated with the risk of breast cancer for patients who are BRCA2 mutation carrier, but was not associated with the risk of breast cancer for BRCA1 mutation carriers [36]. In our comparison by age at diagnosis, we observed strong association of NOTCH2 rs11249433 with increased risk of early onset of breast cancer. The GA heterozygotes were at about 5-fold increased risk of developing breast cancer at younger age compared to those harboring GG genotype. Similar association of this SNP with risk of breast cancer pertaining the age at diagnosis was not observed in women of European ancestry [18, 20]. This discrepancy could be due to other environmental as well as genetic factors and need further investigations.

Our data showed that rs1043994 in NOTCH3 although not significantly associated with breast or colorectal cancer in the overall analysis was having a statistically significant association with colorectal cancers in males. The GA heterozygote males of this SNP were at approximately 2-fold higher risk of developing colorectal cancers compared to GG homozygotes. Colorectal cancer is the predominant cancer in Saudi Arabian males. As cancer risk can be influenced by differential gene expression pattern between men and women as a result of differences in their hormonal and genetic factors, the association of colorectal cancer in men could be attributed to NOTCH3 - rs1043994 variants. Gender related differences in the prognosis of several cancers including colorectal cancer have been reported [37–41]. A link between genetic polymorphism and overall survival in colorectal cancer patients based on gender has been demonstrated in earlier studies [42, 43]. Yagci et al indicated that NOTCH3 rs1043994 is associated with the risk of developing lung cancer in patients of Turkish origin [24]. Associations of NOTCH3-rs1043994 synonymous variant with lacunar infarction and migraine have also been reported in Chinese and German patients, respectively [44, 45].

In Chinese population, while the association of NOTCH2-rs11249433 and NOTCH3-rs1043994 was lacking with breast cancer risk, NOTCH1-rs3124591 was significantly associated with invasive ductal carcinoma and ductal carcinoma in situ [22]. Furthermore, a positive correlation between TC genotype of NOTCH1-rs3124591 and high notch1 protein expression in ductal carcinoma in situ but not in invasive ductal carcinoma was observed [22]. Our data did not show any significant association of NOTCH1-rs3124591 with either CRC or breast cancer. Besides, NOTCH1-rs3124591 is also significantly correlated with nephrotic syndrome risk and alteration in its sensitivity to hormone in Chinese population [46]. A recent study by Yu et al demonstrated that Chinese patients carrying the TT genotype of NOTCH4-rs3830041 had poorer overall survival in contrast to those carrying TC/CC genotype and concluded that rs3830041 variant is an independent predictive marker for prognosis in hepatitis B virus-related hepatocellular carcinoma patients [23]. However, we did not find significant association of NOTCH4-rs3830041 with risk of breast or colorectal cancer in our population.

While there are few reports in the literature on the correlation of the four NOTCH receptor SNPs that we examined on breast cancer, this is the first study to screen these SNPs in colorectal cancers. Screening of larger population of different ethnicity validating our findings on the association of NOTCH2-rs11249433 with breast cancer particularly in younger women and NOTCH3-rs1043994 with colorectal cancer in men would prove beneficial in utilizing these variants as genetic markers for early diagnosis and management of these malignancies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of King Fahad Medical City, Riyadh, and King Khalid University Hospital, Riyadh. The patients/participants provided their written informed consent to participate in this study.
REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J Clin (2021). doi:10.3322/caac.21660
2. Albeshan SM, Mackey MG, Hossain SZ, Alfuraih AA, Brennan PC. Breast cancer epidemiology in Gulf Cooperation Council countries: a regional and international comparison. Clin Breast Cancer (2018) 18(3):e381–e392. doi:10.1016/j.clb.2017.07.006
3. Ferlay JE, Lam F, Colombet M, Mery L, Piñeros M, et al. Global cancer observatory: cancer today. Lyon, France: International Agency for Research on Cancer (2020). Available from: https://gco.iarc.fr/today (Accessed February 21, 2021).
4. Lilyquist J, Ruddy KJ, Vachon CM, Couch FJ. Common genetic variation and breast cancer risk: past, present, and future. Cancer Epidemiol Biomarkers Prev (2018) 27(4):380–94. doi:10.1158/1055-9965.EPI-17-1144
5. Kruk J, Czernecki U. Physical activity and its relation to cancer risk: updating the evidence. Asian Pac J Cancer Prev (2013) 14(7):3993–4003. doi:10.7314/apjcp.2013.14.7.3993
6. Tse G, Edlick GD. Cruciferous vegetables and risk of colorectal neoplasms: a systematic review and meta-analysis. Nutr Cancer (2014) 66(1):128–39. doi:10.1080/01635581.2014.852686
7. Ruiz-Narvaez EA, Lunetta KL, Hong C-C, Haddad S, Yao S, Cheng T-YD, et al. Genetic variation in the insulin, insulin-like growth factor, growth hormone, and leptin pathways in relation to breast cancer in African-American women: the AMBER consortium. NPJ Breast Cancer (2016) 2(1):16034. doi:10.1038/npjbcancer.2016.34
8. Fernández-Lopez JC, Romero-Córdoba S, Rebollar-Vega R, Alfaro-Ruiz LA, Jiménez-Morales S, Beltrán-Anaya F, et al. Population and breast cancer patients’ analysis reveals the diversity of genomic variation of the BRCA genes in the Mexican population. Hum Genomics (2019) 13(1):3. doi:10.1186/s40246-018-0188-9
9. Zhang K, Civan J, Mukherjee S, Patel F, Yang H. Genetic variations in colorectal cancer risk and clinical outcome. World J Gastroenterol (2014) 20(15):4167–77. doi:10.3748/wjg.v20.i15.4167
10. Phipps AI, Passarelli MN, Chan AT, Harrison TA, Jeon J, Hutter CM, et al. Interactions between genetic variants and breast cancer risk factors in the breast and prostate cancer cohort consortium. JNCI J Nat Cancer Inst (2010) 9:113. doi:10.1186/bcr930
11. Savas S, Liu G. Studying genetic variations in cancer prognosis (and risk): a primer for clinicians. Oncologist (2009) 14(7):657–66. doi:10.1634/theoncologist.2009-0042
12. Bray SJ. Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol (2006) 7(9):678–89. doi:10.1038/nrm2009
13. Barr C, Watkins G, Jiang W. The possible correlation of Notch-1 and Notch-2 with clinical outcome and tumour clinicopathological parameters in human breast cancer. Int J Mol Med (2004) 14(5):779–86. doi:10.3892/ijmm.14.5.779
14. Mikaelian I, Blades N, Churchill GA, Fancher K, Knowles BB, Eppig JT, et al. Proteotypic classification of spontaneous and transgenic mammary neoplasms. Breast Cancer Res (2004) 6(6):R668–679. doi:10.1186/bcr930
15. Zhang Y, Li B, Li Z-Z, Zheng P-S. Notch1 regulates the growth of human colon cancers. Cancer (2010) 116(22):5207–18. doi:10.1002/cncr.25449
16. Vinson KE, George DC, Fender AW, Bertrand FE, Sigounas G. The Notch pathway in colorectal cancer. Int J Cancer (2016) 138(8):1835–42. doi:10.1002/ijc.29800
17. Wu G, Chen Z, Li J, Ye F, Chen G, Fan Q, et al. NOTCH4 is a novel prognostic marker that correlates with colorectal cancer progression and prognosis. J Cancer (2018) 9(13):2374–9. doi:10.7150/jcj.26359
18. Thomas G, Jacobs KB, Kraft P, Yeager M, Wacholder S, Cox DG, et al. A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). Nat Genet (2009) 41(5):57–84. doi:10.1038/ng.353
19. Fu Y-P, Edvardsen H, Kaushiva A, Arhancet JP, Howe TM, Kohaar I, et al. NOTCH2 in breast cancer: association of SNP rs11249433 with gene expression in ER-positive breast tumors without TP53 mutations. Mol Cancer (2010) 9:113. doi:10.1186/1476-4598-9-113
20. Campa D, Kaaks R, Le Marchand L, Haiman CA, Travis RC, Berg CD, et al. Interactions between genetic variants and breast cancer risk factors in the breast and prostate cancer cohort consortium. JNCI J Nat Cancer Inst (2011) 103(16):1252–63. doi:10.1093/jnci/djr265
21. Wu S, Cai J, Wang H, Zhang H, Yang W. Association between 1p11-rs11249433 polymorphism and breast cancer susceptibility: evidence from 15 case-control studies. PLoS One (2013) 8(7):e72526. doi:10.1371/journal.pone.0072526
22. Cao YY, Wan GX, Zhao CX, Hu JM, Li L, Liang WH, et al. Notch1 single nucleotide polymorphism rs3124951 is associated with the risk of development of invasive ductal breast carcinoma in a Chinese population. Int J Clin Exp Pathol (2014) 7(7):4286–94.
23. Yu T, Han C, Zhu G, Liao X, Qin W, Yang C, et al. Prognostic value of Notch1 receptors in postsurgical patients with hepatitis B virus-related hepatocellular carcinoma. Cancer Med (2017) 6(7):1587–600. doi:10.1002/cam4.1077
24. Yagci E, Degirmenci I, Ozbayr C, Ak G, Saydam F, Metintas M. Common variants rs3815188 and rs1043994 on Notch3 gene confer susceptibility to lung cancer: a hospital-based case-control study. J Environ Pathol Toxicol Oncol (2019) 38(1):61–8. doi:10.1615/JEnvironPatholToxicolOncol.v2018028403
25. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal (1999) 14(5-6):143–9. doi:10.1016/s1050-3862(99)00019-9
26. Venkatesh V, Nataraj R, Thangaraj GS, Karthikeyan M, Gnanesekaran A, Kagiinelli SB, et al. Targeting Notch signalling pathway of cancer stem cells. Stem Cell Investig. (2018) 5:5. doi:10.21037/sci.2018.02.02
27. Zardawi SJ, Zardawi I, McNeil CM, Millar EKA, McLeod D, Morey AL, et al. High Notch1 protein expression is an early event in breast cancer development and is associated with the HER-2 molecular subtype. Histopathology (2010) 56(3):286–96. doi:10.1111/j.1365-2559.2009.03475.x
28. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell (2000) 100(1):57–70. doi:10.1016/s0092-8674(00)01683-9
29. Schmitt VD, Campbell DA, Sehgal S, Anderson WH, Burns DK, Middleton LT, et al. Pharmacogenetics and disease genetics of complex}

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.por-journal.com/articles/10.3389/pore.2021.616204/full#supplementary-material.
37. Smith RE, Colangelo L, Wieand HS, Begovic M, Wolmark N. Randomized
protocol C-01. J Nat Cancer Inst (2004) 96(15):1128–32. doi:10.1093/inci/djh220
38. Simon MS, Chlebowski RT, Wactawski-Wende J, Johnson KC, Muskovitz A,
differences in colorectal cancer survival: population-based analysis of 164,996
colorectal cancer patients in Germany. PLoS One (2013) 8(7):e68077. doi:10.1371/journal.pone.0068077
39. Majek O, Klatte T, Fujkovic H, de Martino M, Shariat SF. Gender differences in
colon cancer. Cancer Treat Res (2010) 155:15–32. doi:10.1007/978-1-4419-6033-7_2
40. Lucca I, Klatte T, Fujkovic H, de Martino M, Shariat SF. Gender differences in
incidence and outcomes of urothelial and kidney cancer. Nat Rev Urol (2015)
12(10):585–92. doi:10.1038/nruro.2015.232
41. Yoshida Y, Murayama T, Sato Y, Suzuki Y, Saito H, Nomura Y. Gender
differences in long-term survival after surgery for non-small cell lung
Thorac Cardiovasc Surg (2016) 64(6):507–14. doi:10.1055/s-0035-1558995
42. Press OA, Zhang W, Gordon MA, Yang D, Lurje G, Iqbal S, et al. Gender-
differences associated with EGFR polymorphisms in metastatic
colon cancer. Cancer Res (2008) 68(8):3037–42. doi:10.1158/0008-5472.CAN-
07-2718
43. Garufi C, Giacomini E, Torsello A, Sperduti I, Melucci E, Mottolese M, et al.
Gender effects of single nucleotide polymorphisms and miRNAs targeting
clock-genes in metastatic colorectal cancer patients (mCRC). Sci Rep (2016) 6:
34006. doi:10.1038/srep34006
44. Schwaag S, Evers S, Schirmer A, Stögbauer F, Ringelstein E, Kuhlenbäumer G. Genetic variants of the NOTCH3 gene in migraine-A mutation analysis and
gender effects of single nucleotide polymorphisms and miRNAs targeting
colon cancer. Cancer Res (2008) 68(8):3037–42. doi:10.1158/0008-5472.CAN-
07-2718
45. Yoshida Y, Murayama T, Sato Y, Suzuki Y, Saito H, Nomura Y. Gender
differences in long-term survival after surgery for non-small cell lung
cancer. Thorac Cardiovasc Surg (2016) 64(6):507–14. doi:10.1055/s-0035-1558995
46. Yang R, Hong H, Wang M, Ma Z. Correlation between single-nucleotide polymorphisms and miRNAs targeting
clock-genes in metastatic colorectal cancer patients (mCRC). Sci Rep (2016) 6:
34006. doi:10.1038/srep34006
47. Schwag S, Evers S, Schirmacher A, Stögbauer F, Ringelstein E, Kuhlenbäumer G. Genetic variants of the NOTCH3 gene in migraine-A mutation analysis and
gender effects of single nucleotide polymorphisms and miRNAs targeting
colon cancer. Cancer Res (2008) 68(8):3037–42. doi:10.1158/0008-5472.CAN-
07-2718
48. Li Y, Liu N, Chen H, Huang Y, Zhang W. Association of Notch3 single-
nucleotide polymorphisms and miRNAs targeting clock-genes in metastatic colorectal cancer patients (mCRC). Sci Rep (2016) 6:
34006. doi:10.1038/srep34006
49. Yang R, Hong H, Wang M, Ma Z. Correlation between single-nucleotide polymorphisms and miRNAs targeting
clock-genes in metastatic colorectal cancer patients (mCRC). Sci Rep (2016) 6:
34006. doi:10.1038/srep34006
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