Differences of Variable Number Tandem Repeats in XRCC5 Promoter Are Associated with Increased or Decreased Risk of Breast Cancer in BRCA Gene Mutation Carriers

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Ku80 is a subunit of the Ku heterodimer that binds to DNA double-strand break ends as part of the non-homologous end joining (NHEJ) pathway. Ku80 is also involved in homologous recombination (HR) via its interaction with BRCA1. Ku80 is encoded by the XRCC5 gene that contains a variable number tandem repeat (VNTR) insertion in its promoter region. Different VNTR genotypes can alter XRCC5 expression and affect Ku80 production, thereby affecting NHEJ and HR pathways. VNTR polymorphism is associated with multiple types of sporadic cancer. In this study, we investigated its potential association with familial breast cancer at the germline level. Using PCR, PAGE, Sanger sequencing, and statistical analyses, we compared VNTR genotypes in the XRCC5 promoter between healthy individuals and three types of familial breast cancer cases: mutated BRCA1 (BRCA1+), mutated BRCA2 (BRCA2+), and wild-type BRCA1/BRCA2 (BRCAx). We observed significant differences of VNTR genotypes between control and BRCA1+ group (P < 0.0001) and BRCA2+ group (P = 0.0042) but not BRCAx group (P = 0.2185), and the differences were significant between control and cancer-affected BRCA1+ cases (P < 0.0001) and BRCA2+ cases (P = 0.0092) but not cancer-affected BRCAx cases (P = 0.4251). Further analysis indicated that 2R/2R (OR = 1.94, 95%CI = 1.26–2.95, P = 0.0096) and 2R/1R (OR = 1.58, 95%CI = 1.14–2.32, P = 0.0242) were associated with increased risk in cancer-affected BRCA1+ group; 2R/1R (OR = 1.94, 95%CI = 1.14–3.32, P = 0.0242) was associated with increased risk in cancer-affected BRCA2+ group. No correlation was observed for the altered risk between cancer-affected or -unaffected carriers and between different age of cancer diagnosis in cancer-affected carriers. The frequently

Abbreviations: BRCA1, breast cancer 1, early onset; BRCA2, breast cancer 2, early onset; BRCAx, familial breast cancer with wild type BRCA1 and BRCA2; HR, homologous recombination; NHEJ, non-homologous end joining; PAGE, polyacrylamide gel electrophoresis; VNTR, variable number tandem repeat.
observed VNTR association with in BRCA1<sup>+</sup> and BRCA2<sup>+</sup> breast cancer group indicates that VNTR polymorphism in the XRCC5 promoter is associated with altered risk of breast cancer in BRCA1<sup>+</sup> and BRCA2<sup>+</sup> carriers.

**Keywords:** Ku80, XRCC5, promoter, VNTR, familial breast cancer, BRCA1, BRCA2, association

**INTRODUCTION**

Breast cancer is the major cancer type in women. Up to 20% of breast cancer cases have familial genetic background, with multiple family members across generations affected by the disease (1). The discovery of the germline mutations in BRCA1 and BRCA2 confirmed the presence of genetic predisposition for familial breast cancer (2–4). These genes maintain genome stability in normal cells by repairing double-strand breaks mainly through homologous recombination (HR) pathway; their mutated forms lead to genome instability and increased risk for breast cancer development (5). There are two types of DNA double-strand break repair mechanisms: non-homologous end joining (NHEJ) and HR (6). Deficiency in the HR pathway, mainly caused by BRCA germline mutations, is well known to increase the risk of breast cancer (7); however, it is not equally clear whether deficiency in NHEJ pathway can also increase breast cancer risk (8).

Ku is a heterodimer consisting of Ku80 encoded by XRCC5 and Ku70 encoded by XRCC6. Ku recognizes DNA double-strand break ends to initiate the NHEJ pathway, and Ku can also affect the function of the HR pathway by interacting with BRCA1 (9–13). Deletion of XRCC5 in mice leads to increased chromosomal instability, immune deficiency, growth retardation, and cancer (14, 15). Altered expression of XRCC5 promotes oncogenic phenotypes, including hyper proliferation and resistance to apoptosis, genomic instability, and tumorigenesis (16), and has been observed in various types of sporadic cancer, including bladder, breast, colorectal, skin, esophageal, gastric, head, and neck cancer (17–22).

Variable number tandem repeats (VNTRs) are tandem repeat DNA sequences often located in gene regulatory regions that can influence gene expression (23–25). VNTRs follow a Mendelian pattern of inheritance. The XRCC5 promoter contains a VNTR at −160 bp, with a 21-bp repetitive unit (TGCGCATGCTCGCCGGAAATC) hosting a putative Sp1-binding site (26). Studies in Chinese and Iranian populations have demonstrated the presence of VNTR alleles ranging from 0 to 3 21-bp tandem repeats (0R, 1R, 2R, and 3R), with individual genotypes of 0R/0R, 1R/0R, 1R/1R, 2R/0R, 2R/1R, 2R/2R, 3R/0R, 3R/1R, 3R/2R (22, 23). Experimental data indicate that the number of VNTR repeats is inversely related to XRCC5 expression, with an increase in the number of VNTR repeats linked to decreased XRCC5 expression (27–29) (Figure 1A). VNTR polymorphisms in the XRCC5 promoter are associated with sporadic bladder, gastric, and breast cancer (30–32).

Given the transmission pattern of VNTR, the uncertainty regarding the role of NHEJ in familial breast cancer, the presence of VNTR polymorphisms in the XRCC5 promoter, and the association of VNTR polymorphisms with sporadic cancer, we hypothesized that VNTR in the XRCC5 promoter could be involved in familial breast cancer. Therefore, we screened germline VNTR polymorphisms in the XRCC5 promoter in three types of familial breast cancer (BRCA1<sup>+</sup>, BRCA2<sup>+</sup>, and BRCAx). The results showed that certain genotypes of VNTR polymorphisms are associated with the risk of familial breast cancer in BRCA1<sup>+</sup> and BRCA2<sup>+</sup> carriers.

**MATERIALS AND METHODS**

**Study Population**

The familial breast cancer cases used in this study included three subtypes: familial breast cancer with BRCA1 mutation (BRCA1<sup>+</sup>), familial breast cancer with BRCA2 mutation (BRCA2<sup>+</sup>), and familial breast cancer without BRCA1 or BRCA2 mutations (BRCAx). Samples were obtained from the Hereditary Cancer Center at Creighton University (Tables S1–S3 in Supplementary Material). Healthy control samples of age- and gender-matched, de-identified Caucasian individuals were obtained from the Nebraska Biobank of the University of Nebraska Medical Center and The Nebraska Medical Center. The use of patient samples for this study was approved by the Institutional Review Board of Creighton University School of Medicine (00-12265) and of the University of Nebraska Medical Center (718-11-EP). Written and informed consent to participate in the study and to publicate the results was obtained from all subjects.

**Genotyping VNTR Polymorphisms in the XRCC5 Promoter**

PCR amplification, PAGE gel separation, and Sanger sequencing were used to determine VNTR genotype in the XRCC5 promoter of each patient. PCR primer sequences were based on a previously published study (22) with sense primer 5′AGGCGGCTCAAACCAACACAGAC3′ and antisense primer 5′CAAGCGGCAGATAGCAGGAAG3′. The PCR mixture consisted of DNA (20 ng), sense and antisense primers (10 pmol), and GoTaqH DNA polymerase (2 U, Promega). The PCR cycling conditions were 7 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 62°C, and 45 s at 72°C; and a final extension of 7 min at 72°C. An 8% PAGE gel was used to separate PCR products to determine allele type and genotype in each case (3R allele = 287 bp; 2R allele = 266 bp; 1R allele = 245 bp; and 0R allele = 224 bp). Representative products were isolated from PAGE gels and validated by Sanger sequencing.

**VNTR Genotypes in the XRCC5 Promoter of Caucasians**

Data from Iranian and Chinese healthy populations showed that VNTR genotypes in the XRCC5 promoter can vary between ethnic groups (27, 28). To determine whether the data from
these healthy populations can be used as suitable healthy controls for our study in breast cancer of Caucasian cases, we tested the genotypes of 100 healthy local Caucasian individuals and compared these with the genotypes from 535 Caucasian Iranian and 235 Chinese populations (27). The results showed no significant difference in genotypes between the local and Iranian Caucasian populations ($P = 0.3774$) with 2R/2R, 2R/1R, and 1R/1R as the major genotypes, but a significant difference was seen in the genotypes between local Caucasian and Chinese populations ($P < 0.0001$), and Iranian Caucasian and Chinese ($P < 0.0001$), whose genotypes included 2R/2R, 2R/1R, 2R/0R, 1R/1R, 1R/0R, and 0R/0R (Table 1). The 535 Iranian cases were from a Caucasian population living in the Fars province of Iran (27). Because these Iranian cases and our local cases were of the same ethnicity and
there were no significant differences in genotypes between the two groups, the genotypes of the 100 local cases and the 535 Iranian cases were combined to make up the control population for downstream analyses. The combined control group is at Hardy–Weinberg equilibrium ($X^2 = 4.3485$, df = 6, $P = 0.6296$).

### Statistical Analyses

Fisher’s exact test was applied to determine the differences of VNTR polymorphism between the groups of familial breast cancer populations and control population, each type of breast cancer and cancer-affected and -unaffected subgroups within each type of cancer. Both odds ratios and their 95% confidence intervals and $P$-values were computed by using exact methods to keep consistency (33). Benjamini and Hochberg method was used to control the false positive rate at 0.05 (34). Analyses were performed using SAS® software version 9.4 (SAS Institute Inc., Cary, NC, USA).

### RESULTS

#### Samples Used in the Study

$BRCA1^+$ carrier refers to the women who tested positive for a pathogenic $BRCA1$ mutation; $BRCA2^+$ refers to the women who tested positive for a pathogenic $BRCA2$ mutation; and $BRCAx$ refers to the women who tested negative for the mutations in $BRCA1$, $BRCA2$, and p53, with two or more first or second degree relatives affected with primary in situ or invasive breast, ovarian, fallopian tube, or peritoneal cancer, and at least one person must have negative test result. Under each group, the cases were further divided into breast cancer (ovarian cancer)-affected and -unaffected carriers. The average ages at breast cancer diagnosis among the groups were 41.4 ($BRCA1^+$), 43.6 ($BRCA2^+$), and 47.7 ($BRCAx$). The age distributions are consistent with existing data that $BRCA1$ and $BRCA2$ mutation carriers tend to suffer cancer at earlier age. Most of the breast cancers were ductal type and ER-positive; all, but one, of the cases of ovarian cancer were invasive at diagnosis (Table 2).

#### VNTR Genotypes in the XRCC5 Promoter

The four VNTR alleles in the XRCC5 promoter consist of three 21-bp (TGCGCATGCTGCCGGGAATC) tandem repeats (3R), two 21-bp repeats (2R), one 21-bp repeat (1R), and without repeat (0R). The combination of PCR, PAGE, and Sanger sequencing methods provided an effective means to determine VNTR genotypes formed.

### TABLE 1 | Genotype distribution in three ethnical populations.

| Genotype  | Local | Iranian | Chinese |
|-----------|-------|---------|---------|
| 3R/2R     | 0 (0) | 4 (1)   | 0 (0)   |
| 3R/1R     | 1 (1) | 8 (1)   | 0 (0)   |
| 3R/0R     | 1 (1) | 1 (0)   | 0 (0)   |
| 2R/2R     | 16 (16)| 84 (16)| 28 (12)|
| 2R/1R     | 50 (50)| 205 (38)| 57 (24)|
| 2R/0R     | 5 (5) | 29 (5) | 71 (30)|
| 1R/1R     | 22 (22)| 168 (31)| 12 (5)|
| 1R/0R     | 5 (5) | 33 (6) | 37 (16)|
| 0R/0R     | 0 (0) | 3 (1)  | 30 (13)|
| Total     | 100 (100)| 535 (100)| 235 (100)|

| $P$ value  | Local to Iranian: 0.3774 | Local to Chinese: <0.0001 | Iranian to Chinese: <0.0001 |

### TABLE 2 | Summary of the $BRCA1^+$, $BRCA2^+$, and $BRCAx$ carriers used in the study*.

| Items                      | $BRCA1^+$ | $BRCA2^+$ | $BRCAx$ |
|----------------------------|-----------|-----------|---------|
| Unaffected cases           | 60        | 29        | 11      |
| Average current age        | 56.9 ± 14.4| 49.1 ± 14.4| 66.4 ± 15.8|
| Affected cases             | 166       | 69        | 89      |
| Average age at diagnosis   | 41.4 ± 10.8| 43.6 ± 10.3| 47.7 ± 12.0|
| Proband                    | 38        | 15        | 62      |
| Non-proband                | 128       | 54        | 27      |
| Breast cancer              | 166       | 69        | 89      |
| ER                         | 22(+43)(−)| 17(+8)(−) | 27(+8)(−)|
| Unknown                    | 101       | 44        | 56      |
| PR                         | 17(+45)(−)| 15(+9)(−) | 21(+9)(−)|
| Unknown                    | 104       | 45        | 59      |
| HER2/neu                   | 4(+10)(−) | 3(+4)(−)  | 6(+17)(−)|
| Unknown                    | 152       | 62        | 66      |
| Lymph nodes                | 38(+54)(−)| 18(+23)(−)| 16(+16)(−)|
| Unknown                    | 74        | 30        | 57      |
| Left                       | 56        | 17        | 24      |
| Right                      | 55        | 25        | 27      |
| Bilateral                  | 40        | 19        | 8       |
| Unknown                    | 15        | 8         | 30      |
| Ovarian cancer             | 21        | 5         | 15      |
| Left                       | 3         | 3         | 1       |
| Right                      | 4         | 1         | 2       |
| Bilateral                  | 14        | 4         | 12      |
| Fallopian tube             | 1         | 1         |         |
| Lymph nodes                | 7(+10)(−) | 1(+7)     | 2(+3)(−)|
| Unknown                    | 4         | 4         | 10      |
| Carcinoma, not specified   | 3         | 4         |         |
| Clear cell adenocarcinoma  | 1         | 1         |         |
| Papillary adenocarcinoma   | 2         | 2         | 1       |
| Adenocarcinoma (cystadenocarcinoma) | 9     | 1         | 1       |
| Endometrioid adenocarcinoma| 2         |           |         |
| Serous (cyst) adenocarcinoma| 5       | 1         | 3       |
| Dysgerminoma               | 1         |           |         |
| Unknown                    | 1         | 5         |         |
| In situ                    | 20        | 5         | 9       |
| Invasive                   | 1         | 1         |         |
| Unknown                    | 1         |           | 5       |

*Some number in categories may not add up to the total due to incompleteness of tested cases.
by the four alleles. Figure 1B shows the genotypes of homozygotes (1R/1R and 2R/2R) and heterozygotes (3R/2R, 3R/1R, 2R/1R, 2R/0R, and 1R/0R), and Figure 1C shows the sequences of the 21-bp repeats from the homozygotes (1R/1R and 2R/2R).

**VNTR Genotype Distribution, BRCA Pre disposition, and Cancer Status**

We compared the VNTR genotype distributions in the XRCC5 promoter between three types of familial breast cancer: BRCA1+, BRCA2+, and BRCAx (Tables S1–S3 in Supplementary Material). The results show that the BRCA1+ and BRCA2+ groups differed significantly from the control group (BRCA1+ group: \( P < 0.0001 \); BRCA2+ group: \( P = 0.0042 \)), but no difference was observed between the BRCAx groups and control group (\( P = 0.1308 \)) (Table 3). To test whether different VNTR genotype distribution exists relating to disease status, the three types of familial breast cancer were divided into breast cancer-affected and breast cancer-unaffected subgroups and further compared each subgroup with the control group. The results show that the differences were only present between the cancer-affected subgroups in both control and carrier population, we removed 3R/2R, 3R/1R, 2R/0R, and 0R/0R but focused on the 2R/2R, 2R/1R, 1R/1R, and 1R/0R as they contributed most of the cases. The results show that the decreased risk in cancer-affected BRCA1+ (\( P = 0.2216 \) and BRCA2+ (cancer-affected: \( P = 0.0092 \), cancer-unaffected: \( P = 0.2748 \)) did not have a significant difference with the control group. The results show that the differences were only present between the cancer-affected subgroups and further compared each subgroup with the control group. The results show that the differences were only present between the cancer-affected subgroups and further compared each subgroup with the control group. The results show that the differences were only present between the cancer-affected subgroups and further compared each subgroup with the control group. The results showed that

1. BRCA1+ group. 2R/2R (OR = 1.94, 95%CI = 1.26–2.95, \( P = 0.0096 \)) and 2R/1R (OR = 1.58, 95%CI = 1.11–2.26, \( P = 0.0388 \)) were associated with increased risk of breast cancer in cancer-affected BRCA1+ group, and 1R/1R (OR = 0.55, 95%CI = 0.35–0.84, \( P = 0.0196 \)) and 1R/0R (OR = 0, 95%CI = 0–0.29, \( P = 0.0012 \)) were associated with the decreased risk in cancer-affected BRCA1+ group;
2. BRCA2+ group. 2R/2R (OR = 1.94, 95%CI = 1.14–3.32, \( P = 0.0242 \)) was associated with increased risk in cancer-affected BRCA2+ group. 2R/2R, 1R/1R, and 1R/0R had no association with the risk of breast cancer in cancer-affected BRCAx group;
3. BRCAx group. 2R/2R, 2R/1R, 1R/1R, and 1R/0R had no association with the risk of breast cancer in cancer-affected BRCAx group.

**DISCUSSION**

Gene regulatory regions have long been considered a potential source of "missing heritability" in cancer (35, 36). Our study provides evidence showing that VNTR polymorphisms in the

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**Table 3** Genotype distribution in three types of familial breast cancer.

| Genotype | Control | BRCA1+ | BRCA2+ | BRCAx |
|----------|---------|--------|--------|-------|
| Total    | 635 (100) | 226 (100) | 98 (100) | 100 (100) |
| 3R/2R    | 4 (1)    | 0 (0)   | 0 (0)   | 0 (0)   |
| 3R/1R    | 9 (1)    | 2 (1)   | 0 (0)   | 0 (0)   |
| 3R/0R    | 2 (0)    | 0 (0)   | 0 (0)   | 0 (0)   |
| 2R/2R    | 100 (16) | 61 (27) | 27 (28) | 23 (23) |
| 2R/1R    | 255 (40) | 113 (50) | 51 (52) | 48 (48) |
| 2R/0R    | 34 (5)   | 4 (2)   | 1 (1)   | 3 (3)   |
| 1R/1R    | 190 (30) | 45 (20) | 17 (17) | 19 (19) |
| 1R/0R    | 38 (6)   | 1 (0)   | 2 (2)   | 7 (7)   |
| 0R/0R    | 3 (0)    | 0 (0)   | 0 (0)   | 0 (0)   |
| \( P \) value | <0.0001 | 0.0042 | 0.2185 |

**Table 4** Genotypes between cancer-affected and unaffected familial breast cancer.

| Genotype | Control | BRCA1+ | No cancer | BRCA2+ | Cancer | No cancer | BRCAx | Cancer | No cancer |
|----------|---------|--------|-----------|--------|--------|-----------|-------|--------|-----------|
| Total    | 635 (100) | 166 (100) | 60 (100) | 69 (100) | 29 (100) | 89 (100) | 11 (100) |
| 3R/2R    | 4 (1)    | 0 (0)   | 0 (0)    | 0 (0)   | 0 (0)   | 0 (0)    | 0 (0)   |
| 3R/1R    | 9 (1)    | 2 (1)   | 0 (0)    | 0 (0)   | 0 (0)   | 0 (0)    | 0 (0)   |
| 3R/0R    | 2 (0)    | 0 (0)   | 0 (0)    | 0 (0)   | 0 (0)   | 0 (0)    | 0 (0)   |
| 2R/2R    | 100 (16) | 44 (27) | 17 (28)  | 17 (25) | 10 (34) | 20 (22)  | 3 (27)  |
| 2R/1R    | 255 (40) | 85 (51) | 28 (47)  | 39 (57) | 12 (41) | 41 (46)  | 6 (64)  |
| 2R/0R    | 34 (5)   | 3 (2)   | 1 (2)    | 0 (0)   | 1 (5)   | 3 (3)    | 0 (0)   |
| 1R/1R    | 190 (30) | 32 (19) | 13 (22)  | 13 (19) | 4 (14)  | 18 (20)  | 1 (9)   |
| 1R/0R    | 38 (6)   | 0 (0)   | 1 (2)    | 0 (0)   | 2 (7)   | 7 (8)    | 0 (0)   |
| 0R/0R    | 3 (0)    | 0 (0)   | 0 (0)    | 0 (0)   | 0 (0)   | 0 (0)    | 0 (0)   |
| \( P \) value | <0.0001 | 0.2216 | 0.0092    | 0.2748  | 0.4251  | 0.5664  |
TABLE 5 | Comparison between affected and unaffected group.

| Genotype | Affected case | Unaffected case | Odds ratio | 95% CI | P-value | Adjusted |
|----------|---------------|----------------|------------|--------|---------|----------|
| BRCA1+   |               |                |            |        |         |          |
| 2R/2R    | 45            | 16             | 0.9299     | 0.46–1.96 | 0.8637   | 0.9422   |
| 2R/1R    | 87            | 26             | 1.265      | 0.66–2.42 | 0.5403   | 1        |
| 1R/1R    | 32            | 13             | 0.7906     | 0.37–1.79 | 0.5664   | 1        |
| 1R/0R    | 0             | 0              | 0          | 0–64.08  | 0.2522   | 1        |
| BRCA2+   |               |                |            |        |         |          |
| 2R/2R    | 17            | 10             | 0.769      | 0.27–2.26 | 0.6281   | 0.9422   |
| 2R/1R    | 39            | 12             | 1.8417     | 0.70–4.91 | 0.1904   | 1        |
| 1R/1R    | 13            | 4              | 1.3929     | 0.38–6.45 | 0.7707   | 1        |
| 1R/0R    | 0             | 2              | 0          | 0–1.44   | 0.0854   | 1        |
| BRCAx    |               |                |            |        |         |          |
| 2R/2R    | 20            | 3              | 0.77       | 0.19–3.19 | 0.7118   | 0.9350   |
| 2R/1R    | 41            | 6              | 0.71       | 0.20–2.50 | 0.5951   | 1        |
| 1R/1R    | 18            | 1              | 2.54       | 0.30–21.12 | 0.6850   | 1        |
| 1R/0R    | 7             | 0              | Infinity   | 0.23–infinity | 1        | 1        |

TABLE 6 | Association of VNTR genotypes in XRCC5 promoter with familial breast cancer-affected and -unaffected groups.

| Genotype | Control | Affected case | Odds ratio | 95% CI | P-value | Adjusted | Unaffected case | Odds ratio | 95% CI | P-value | Adjusted |
|----------|---------|---------------|------------|--------|---------|----------|----------------|------------|--------|---------|----------|
| BRCA1+   |         |               |            |        |         |          |                |            |        |         |          |
| 2R/2R    | 100     | 45            | 1.94       | 1.26–2.95 | 0.0016  | 0.0096   | 16             | 2.09      | 1.05–3.97 | 0.0221  | 0.1326   |
| 2R/1R    | 255     | 87            | 1.58       | 1.11–2.26 | 0.0087  | 0.0388   | 26             | 1.25      | 0.69–2.23 | 0.3190  | 0.4785   |
| 1R/1R    | 190     | 32            | 0.55       | 0.35–0.84 | 0.0049  | 0.0196   | 13             | 0.69      | 0.33–1.35 | 0.2906  | 0.4978   |
| 1R/0R    | 38      | 0             | 0          | 0–0.29   | 0.0001  | 0.0012   | 1              | 0.28      | 0.01–1.73 | 0.2414  | 0.5794   |
| BRCA2+   |         |               |            |        |         |          |                |            |        |         |          |
| 2R/2R    | 100     | 17            | 1.75       | 0.97–3.15 | 0.0865  | 0.1038   | 10             | 2.82      | 1.13–6.58 | 0.0174  | 0.2088   |
| 2R/1R    | 255     | 39            | 1.94       | 1.14–3.32 | 0.0101  | 0.0242   | 12             | 1.05      | 0.45–2.38 | 1       | 1        |
| 1R/1R    | 190     | 13            | 0.54       | 0.27–1.04 | 0.0880  | 0.0907   | 4              | 0.37      | 0.09–1.11 | 0.0632  | 0.1896   |
| 1R/0R    | 38      | 0             | 0          | 0–0.73   | 0.0427  | 0.0641   | 2              | 1.16      | 0.13–4.93 | 0.6916  | 0.7545   |
| BRCAx    |         |               |            |        |         |          |                |            |        |         |          |
| 2R/2R    | 100     | 20            | 1.55       | 0.90–2.67 | 0.1101  | 1        | 3              | 2.01      | 0.52–7.69 | 0.3945  | 0.5257   |
| 2R/1R    | 255     | 41            | 1.27       | 0.84–1.82 | 0.2882  | 0.3096   | 6              | 1.79      | 0.54–9.02 | 0.3654  | 0.5481   |
| 1R/1R    | 190     | 18            | 0.59       | 0.35–1.02 | 0.0683  | 0.3168   | 1              | 0.23      | 0.03–1.84 | 0.1885  | 0.5655   |
| 1R/0R    | 38      | 7             | 1.34       | 0.58–3.10 | 0.4913  | 1        | 0              | 0        | 0–4.75   | 1       | 1        |

XRCC5 promoter is associated with risk of familial breast cancer with BRCA1+ and BRCA2+ predisposition. Data from sporadic breast cancer showed that 2R/1R was not associated (OR = 1.09, 95%CI = 0.78–1.53, P = 0.595), but 0R/0R was associated with the disease (OR = 9.55, 95%CI = 1.19–76.6, P = 0.034) (31). The different results suggest that the association of VNTR polymorphisms in the XRCC5 promoter differs between familial breast cancer and sporadic breast cancer.

For the BRCA1+ and BRCA2+ groups, the results can be explained by synergistic roles between Ku80 and BRCA1/BRCA2 in maintaining genome stability through the NHRJ and HR pathways (37–39). Altered expression of Ku80 can disturb the synergy resulting in increased breast cancer risk in BRCA mutation carriers. The results also suggest that genotype 1R/1R and 1R/0R can reduce the risk of breast cancer in BRCA1+ carriers. Based on current knowledge, it is difficult to relate VNTR polymorphisms to BRCAx familial breast cancer, as genetic predisposition in this heterogeneous group of familial breast cancer remains to be determined. We did not observe a relationship between age at onset of disease, cancer status, and VNTR polymorphism. This could be due to the weaker influence by the VNTR polymorphism compared with that of the BRCA mutation predisposition. Alternatively, it could be due to the limited sample size used in the study, which restricts the statistical power to detect the potential significance. Further studies with larger sample size will help to address the issue.

In summary, our study indicates that 2R/2R and 2R/1R were significantly associated with increased risk, and 1R/1R and 1R/0R were significantly associated with the decreased risk of BRCA1+ breast cancer, whereas 2R/1R was significantly associated with the increased risk of BRCA2+ breast cancer.

AUTHOR CONTRIBUTIONS

JC and BD performed the genotyping and Sanger sequencing experiments; YK analyzed the genotyping data; JC and JL
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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fonc.2016.00092
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Conflict of Interest Statement: The authors declared that the research was conducted in the absence of any commercial and financial relationships that could be constructed as a potential conflict of interest.

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