Homologous recombination DNA repair gene RAD51, XRCC2 & XRCC3 polymorphisms and breast cancer risk in South Indian women

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Abstract

Background
Homologous recombination repair (HRR) accurately repairs the DNA double-strand breaks (DSBs) and is crucial for genome stability. Genetic polymorphisms in crucial HRR pathway genes might affect genome stability and promote tumorigenesis. Up to our knowledge, the present study is the first to investigate the impact of HRR gene polymorphisms on BC development in South Indian women. The present population-based case-control study investigated the association of polymorphisms in three key HRR genes (XRCC2-Arg188His, XRCC3-Thr241Met and RAD51-G135C) with BC risk.

Materials and methods
Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for genotyping the HRR variants in 491 BC cases and 493 healthy women.

Results
We observed that the XRCC3 Met allele was significantly associated with BC risk [OR: 1.27 (95% CI: 1.02–1.60); p = 0.035]. In addition, the homozygous mutant (C/C) genotype of RAD51-G135C variant conferred 2.19 fold elevated risk of BC [OR: 2.19 (95% CI: 1.06–4.54); p = 0.034]. Stratified analysis of HRR variants and BC clinicopathological features revealed that the XRCC3-Thr241Met and RAD51-G135C variants are associated with BC progression. Combined SNP analysis revealed that the individuals with RAD51-C/C, XRCC2-Arg/Arg, and XRCC3-Thr/Thr genotype combination have three-fold increased BC risk.
The present study imparts additional evidence that genetic variants in crucial HRR pathway genes might play a pivotal role in modulating BC risk in South Indian women.
in exon 7, which results in Thr to Met amino acid substitution at codon 241. Additionally, individuals carrying the Met allele had increased DNA adduct levels in the lymphocyte DNA [16, 17]. Moreover, in vitro studies suggested that the XRCC3-241Met variant increased an individual's cancer risk [18]. Besides, Song et al. conducted a meta-analysis and highlighted that the Thr241Met variant was significantly associated with a higher risk of radiation-induced early adverse outcomes, as well as specific detrimental effects such as mucositis and acute skin toxicity [19].

Last decade, there have been conflicting reports regarding the impact of the HRR gene polymorphisms on BC risk [20–26]. Besides, there is a paucity of information regarding the impact of HRR gene polymorphisms on South Indian women’s BC etiology. Hence, we conducted this population-based case-control study to evaluate the impact of XRCC2-Arg188His, XRCC3-Thr241Met, and RAD51-G135C variants with BC risk in South Indian women.

Materials and methods

Study subjects

The present study investigated 984 subjects, which included 491 histopathologically confirmed breast cancer cases and 493 healthy women from South India. The institutional ethics committee of Dr. G.V.N Cancer Institute (ECR/436/INST/TN/2013) and MMHRC (ECR/398/INST/TN/2013/RR-16) approved the present study. The samples were collected following the tenets of the Declaration of Helsinki and its later amendments. Written informed consent was obtained from the study participants. Peripheral blood of BC patients was collected from the medical oncology department of Dr. G.V.N Cancer Institute and Meenakshi Mission Hospital & Research Centre between June 2017 and January 2020. Blood samples of healthy (cancer-free) women, who are age and ethnicity matched to cases and without a family history of cancer, were collected during the same period. The surgically resected primary tumors of the BC patients were graded according to the Scarf-Bloom-Richardson grading system and staged based on the American Joint Committee of Cancer (AJCC) system. Clinico-pathological characteristics of the BC patients such as menopausal status, age at disease onset, hormonal receptor status, tumor grade, tumor stage, and metastasis extent were noted with the help of a medical oncologist.

Genomic DNA extraction

Antecubital venepuncture was performed to draw 3-5ml of venous blood from the study participants in a commercially available sterile K2-EDTA coated vacutainer (BD Vacutainer®, Franklin Lakes, USA). The genomic DNA was extracted from the whole blood samples using the HiPurA SPP blood DNA isolation kit (HiMedia™, Mumbai, India) following the manufacturer’s protocol. The isolated DNA was quantitatively assessed for purity using the NanoDrop 2000™ spectrophotometer (Thermo Fischer Scientific, USA). All the genomic DNA samples were stored at -20°C until further analysis.

Genotyping

PCR–RFLP (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) method was used for genotyping XRCC2-Arg188His, XRCC3-Thr241Met, and RAD51-G135C variants. The PCR reactions were carried out in a final volume of 25μl reaction mixture comprising 12.5μl of 2x GoTaq Green master mix (Promega, Madison, USA), 0.5μl of each primer (10μM), 100-150ng genomic DNA, and nuclease-free water. PCR reactions were carried out using T100™ thermal cycler (Bio-Rad, CA, USA). The PCR amplicons were run on 1%
ethidium bromide-stained agarose gel and visualized using Bio-Rad XR™ gel documentation system (Bio-Rad, CA, USA). The list of primers, annealing temperature, and RFLP conditions utilized is given in Table 1. The investigated variants’ genotypes were determined by performing electrophoresis of the digested PCR products in ethidium bromide-stained 4% agarose gel and visualized using a gel documentation system (Bio-Rad, CA, USA). To assess the genotyping quality, genotyping was repeated in random 10% of the samples, and the results were 100% concordant.

**Statistical analysis**

With respect to the SNPs investigated, the genotypes of the controls were assessed for their agreement with Hardy-Weinberg equilibrium (HWE) using the \( \chi^2 \) goodness of fit test. Odds ratio (OR) and 95% confidence interval (CI) were determined by performing unconditional logistic regression analysis using SNPStats online software (https://www.snpstats.net/). Stratified analysis was carried out between the investigated SNPs and clinicopathological features of BC cases to evaluate SNPs’ role in disease progression. P-value <0.05 was considered as statistically significant. Multifactor-dimensionality reduction (MDR) analysis and interaction dendrogram was constructed using the MDR software package (MDR 3.0.2) to evaluate the impact of gene-gene interaction on BC risk. The best interaction model was selected based on the highest cross-validation consistency (CVC) and testing balance accuracy (TBA). Further, STRING software was used to visualize protein-protein interaction (https://string-db.org/).

**Results**

**Characteristics of the study population**

The present study comprised of 491 breast cancer cases and 493 healthy women as controls. The mean age of BC onset in the patients was 52.1±10.99 yrs. The clinicopathological features of BC cases are summarized in Table 2.

**Hardy-Weinberg Equilibrium (HWE) test**

The observed genotype frequency for the studied polymorphic loci were in accordance with Hardy-Weinberg equilibrium in the controls.

Table 1. PCR conditions, PCR primers and RFLP pattern.

| Gene; SNP | PCR Primers | Annealing Temperature | Restriction enzyme | RFLP pattern |
|-----------|-------------|-----------------------|-------------------|--------------|
| XRCC2;    | F: 5’- TGTAGTCACCCATCTCTGC -3’ | 58°C | HphI | Arg/Arg: 290 bp |
| rs3218536; (G>A) | R: 5’- AGTTGCTGCCATGCTTACA -3’ | | Arg/His: 290 bp, 148 bp & 142 bp | |
| XRCC3;    | F: 5’- GCCTGGTGGTCACTCGACTC -3’ | 61°C | NcoI | Thr/Thr: 136 bp |
| rs861539; (C>T) | R: 5’-ACAGGGCTCTGGAAGGACTCAGCT CAGGCACC -3’ | | Thr/Met: 136 bp, 97 bp & 39 bp | |
| RAD51;    | F: 5’- TGGGAACTGCATCTCTGCTG -3’ | 60°C | MvaI | G/G: 86 bp & 71 bp |
| rs1801320; (G>C) | R: 5’- GCCCTCCTCCTCAGCG -3’ | | G/C: 157 bp, 86 bp & 71 bp | |

F: Forward Primer; R: Reverse Primer; bp: Base pair

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Allele and genotype distribution of XRCC2-Arg188His, XRCC3-Thr241Met, and RAD51-G135C variants

XRCC2-Arg188His variant analysis. Genotype frequency distribution of the XRCC2-Arg188His variant (Arg/Arg, Arg/His and His/His genotypes) was 79.9%, 19.3% and 0.8% in controls and 76.6%, 21.6% and 1.8% in BC cases, respectively. However, no significant association was observed between the genotype and allele frequency distribution of the XRCC2-Arg188His variant in BC cases and controls (Table 3) (S1 File).

XRCC3-Thr241Met variant analysis. The genotype (Thr/Thr, Thr/Met and Met/Met) frequency distribution of the XRCC3-Thr241Met variant was found to be 69.4%, 27.2% and 3.4% in the controls and 63.1%, 32.2% and 4.7% in BC cases respectively (Table 3). A marginal association was observed between the heterozygous genotype and BC risk, where the Thr/Met genotype conferred a 1.30-fold elevated BC risk; however, the association was insignificant (p > 0.05). Under the dominant model, we observed that the Thr/Met + Met/Met genotype conferred an 1.32-fold elevated risk of BC development (OR:1.32 [95% CI: 1.01–1.72]; p = 0.038). Similarly, the allele frequency distribution of the Thr and Met alleles were found to be 83% and 17% in the controls and 79.2% and 20.8% in the BC cases. Interestingly, the Met allele was significantly associated with BC risk [OR:1.27 (95% CI: 1.02–1.60); p = 0.035] in South Indian women (Table 3).

Table 2. Demographic and clinicopathological characteristics of the study subjects.

| Characteristics            | BC cases n (%) | Controls n (%) |
|----------------------------|----------------|----------------|
| Age in years (mean±S.D)    | 52.51±10.99    | 51.40±14.48    |
| Menopausal Status          |                |                |
| Pre-menopause              | 148 (30.1)     | 157 (31.8)     |
| Post-menopause             | 343 (69.9)     | 336 (68.2)     |
| Molecular Subtype          |                |                |
| Luminal                    | 292 (59.4)     |                |
| Her2 enriched              | 102 (20.8)     |                |
| TNBC                       | 97 (19.8)      |                |
| Tumor Stage                |                |                |
| Early (T1+T2)              | 305 (62.1)     |                |
| Advanced (T3+T4)           | 186 (37.9)     |                |
| Histological Grade         |                |                |
| Low (G1)                   | 81 (16.5)      |                |
| High (GII+GIII)            | 410 (83.5)     |                |
| Metastasis                 |                |                |
| Positive                   | 120 (24.4)     |                |
| Negative                   | 371 (75.6)     |                |
| Estrogen Receptor          |                |                |
| Positive                   | 278 (56.6)     |                |
| Negative                   | 213 (43.4)     |                |
| Progesterone Receptor      |                |                |
| Positive                   | 212 (43.2)     |                |
| Negative                   | 279 (56.8)     |                |
| HER2/neu                   |                |                |
| Positive                   | 204 (41.5)     |                |
| Negative                   | 287 (58.5)     |                |

n: number; HER2: Human Epidermal Growth Factor Receptor 2; TNBC: Triple-Negative Breast Cancer.

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RAD51-G135C variant analysis. The frequency of G/G, G/C, and C/C genotypes of the RAD51-G135C variant in the controls were found to be 75.9%, 21.9%, and 2.2%, whereas it was observed to be 75.8%, 19.3%, and 4.9% in BC cases, respectively. We noticed that the homozygous mutant (C/C) genotype conferred 2.19-fold elevated risk of developing BC [OR: 2.19 (95% CI: 1.06–4.54); p = 0.034]. We additionally observed that the C/C genotype conferred 2.25-fold [OR: 2.25 (95% CI: 1.09–4.65); p = 0.023] elevated risk of BC under the recessive inheritance model (G/G+G/C vs. C/C) (Table 3).

### Association between HRR gene polymorphisms and BC clinicopathological characteristics

To evaluate the association between the HRR gene polymorphisms and various BC clinicopathological features, BC patients were stratified based on their genotypes and clinicopathological characteristics. Pertaining to the XRCC3 Thr241Met variant, the heterozygous (Thr/
Met) genotype reduced the risk of developing higher-grade tumors [OR: 0.58 (95% CI: 0.35–0.95); p = 0.031] (Table 4).

However, in contrast, the heterozygous (Thr/Met) genotype was observed to elevate the risk of BC development in women with elevated BMI [OR: 1.66 (95% CI: 1.09–2.54); p = 0.019]. Similarly, the homozygous mutant (Met/Met) genotype was associated with the development of pre-menopausal BC [OR: 2.47 (95% CI: 1.05–5.80); p = 0.039].

Table 4. Evaluation of XRCC3 and RAD51 variants with BC patients’ clinicopathological features.

| CLINICAL VARIABLES       | XRCC3 Thr241Met | RAD51 G135C |
|--------------------------|-----------------|-------------|
| Tumor grade              |                 |             |
| High/Low                 | 266/44          | 123/35      |
| OR (95% CI)              | Reference       | 0.58 (0.35–0.95) |
| p-value                  | 0.031           | 0.466       |
| Tumor Stage              |                 |             |
| III+IV/II+I              | 116/194         | 61/97       |
| OR (95% CI)              | Reference       | 1.05 (0.71–1.56) |
| p-value                  | 0.802           | 0.870       |
| HER2/neu status          |                 |             |
| +ve/-ve                  | 136/174         | 58/100      |
| OR (95% CI)              | Reference       | 0.74 (0.50–1.10) |
| p-value                  | 0.138           | 0.971       |
| PR Status                |                 |             |
| -ve/+ve                  | 134/176         | 67/91       |
| OR (95% CI)              | Reference       | 0.97 (0.66–1.42) |
| p-value                  | 0.865           | 0.406       |
| Metastasis               |                 |             |
| +ve/-ve                  | 76/234          | 38/120      |
| OR (95% CI)              | Reference       | 0.98 (0.62–1.52) |
| p-value                  | 0.912           | 0.204       |
| Age at onset (years)     |                 |             |
| ≤40 / >40                | 40/270          | 26/132      |
| OR (95% CI)              | Reference       | 1.33 (0.78–2.27) |
| p-value                  | 0.298           | 0.085       |
| BMI                      |                 |             |
| 3+4 / 2+1                | 196/114         | 117/41      |
| OR (95% CI)              | Reference       | 1.66 (1.09–2.54) |
| p-value                  | 0.019           | 0.147       |
| Menopausal status        |                 |             |
| Pre/Post                 | 84/226          | 53/105      |
| OR (95% CI)              | Reference       | 1.36 (0.89–2.05) |
| p-value                  | 0.148           | 0.039       |

p-value < 0.05 is considered as significant and are highlighted in bold; OR: Odds ratio; CI: Confidence interval; BMI: Body Mass Index: 4–Obese; 3–Overweight; 2-Normal weight; 1–Underweight; ER: Estrogen Receptor; PR: Progesterone Receptor; HER2: Human Epidermal Growth Factor Receptor 2.

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Analysis of RAD51 G135C variant and BC clinicopathological features revealed that the heterozygous (G/C) genotype reduced BC risk in women with elevated BMI (overweight and obese) [OR: 0.57 (95% CI: 0.36–0.91); p = 0.017]. On the other hand, the homozygous mutant (C/C) genotype was observed to confer an elevated risk of metastasis in women carrying the C/C genotype [OR: 2.41 (95% CI: 1.03–5.62); p = 0.042] (Table 4).

Concerning the XRCC2 Arg188His variant and clinicopathological characteristics, we did not observe a significant association between various BC clinicopathological characteristics and Arg188His polymorphism in the breast cancer patients (S1 Table).

MDR analysis
The combined effect of HRR gene variants (XRCC2-Arg188His, XRCC3-Thr241Met and RAD51-G135C) on BC risk was evaluated using MDR analysis. MDR evaluates the effect of SNP-SNP interaction on the risk of developing a multi-factorial disease such as breast cancer. MDR divides the data into a training dataset (9/10) and an independent testing dataset (1/10). A higher TBA value indicates that the observed interaction accurately predicts the case-control status. Moreover, a TBA score greater than 0.5 indicates that the interaction combination observed is not by chance, and a score of 1.00 highlights that the observed interaction combination is the best [27]. Furthermore, the model that has the highest TBA and CVC can be identified as the best interaction model. Fig 1 represents the various models predicted by MDR.

In the present study, MDR analysis revealed that among the different models, the interaction between XRCC3-Thr241Met and RAD51-G135C variants were observed to be the best interacting model under the two-loci model (TBA: 0.538; CVC: 10/10). Further investigation of the combinatorial impact of HRR variants on BC risk showed that the XRCC2-Arg/Arg, XRCC3-Thr/Thr, and RAD51-C/C genotype combination was elevated in BC cases compared to controls, thereby conferring elevated risk of BC [OR: 3.29 (95% CI: 1.17–9.23); p = 0.024] (Table 5). Fig 2 depicts the first ten protein partners that interact with XRCC2, XRCC3, and RAD51, which includes BRCA1, BRCA2, and RAD51D proteins. The STRING database collects and integrates all the publicly available protein-protein interaction sources and aids in the visualization of both direct (physical) and indirect (functional) interactions [28].

Discussion
Screening for certain commonly occurring polymorphisms has advanced our understanding of the crucial role played by genetics in BC predisposition. Moreover, various studies have reported that subtle variation in DNA repair capacity caused by the combination of low-penetrant genes and other influential factors such as environment modulates BC risk. Also, defects in DNA DSB repair has been identified as a common denominator for mammary carcinogenesis. Moreover, the RAD51 gene family, including XRCC2, XRCC3, and RAD51, is highly polymorphic in nature [29]. Previous reports on the impact of HRR pathway gene variations from various ethnicities have yielded inconsistent results. Hence, the present study aims to clarify the role of genetic variants in crucial HRR genes (XRCC2, XRCC3, and RAD51) towards BC development.

In the present study, we found that the XRCC2 Arg188His variant was not associated with BC predisposition in South Indian women. In line with our report, various studies observed a similar lack of association in Pakistani [30], Caucasian [26], Portuguese [31], African-American and white [32] women. Similarly, various meta-analysis studies investigating the role of the XRCC2 Arg188His variant on BC development reported that the XRCC2 Arg188His variant was not directly associated with BC predisposition [33–35]. Interestingly, a study by Silva
et al. [31] highlighted that individuals who have never-breast fed and are heterozygous (Arg/His) for the XRCC2 rs3218536 variant had reduced risk for BC.

Fig 1. MDR analysis. Each cell depicts the number of BC cases on the left and the number of controls on the right. A high-risk genotype combination is given in dark grey cells, and a low-risk genotype combination is given in light grey cells. (a) Single-loci model representing cases and controls based on XRCC3 (Thr241Met) variant, (b) two-loci model depicting cases and controls classified based on two SNPs (XRCC3 -Thr241Met and RAD51 –G135C), (c) three-loci model depicting cases and controls classified based on three SNPs, (d) interaction dendrogram revealed that the investigated HRR variants were found to have a redundant effect on BC development.

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Investigating the role of XRCC3 Thr241Met variant and BC risk, we observed that the Met allele was associated with BC risk. However, in the present study, we found that the heterozygous (Thr/Met) and homozygous mutant (Met/Met) genotypes were not significantly associated with BC risk. A recent meta-analysis study based on 55 case-control studies on XRCC3 Thr241Met variant and BC risk concluded that the XRCC3 Thr241Met variant was associated with BC risk in Arabian and Asian populations [36]. Additionally, another meta-analysis concluded that the XRCC3 Thr241Met variant was associated with a weakly elevated BC risk [37]. Similarly, another study on Thai [38] and South American [24] women reported that the 241Met carriers were at elevated BC risk. Furthermore, a study by Santos et al. [39] additionally observed that the XRCC3 Thr241Met variant was slightly associated with an increased risk of BC in individuals with elevated chromosomal damage. However, reports from certain ethnicities have observed a lack of association between XRCC3 Thr241Met variant and BC risk [25, 26, 40–43].

Interestingly, various studies reported that the RAD51 G135C variant modified BC risk in BRCA2 mutation carriers [44, 45]. Antoniou et al. [44] suggested that the RAD51 G13C variant located in the 5'UTR region might also affect alternate splicing. Thus, the RAD51 135C allele might cause an overall decrease in RAD51 protein abundance. The present study investigated the role of RAD51 G135C variant and BC risk, and we observed that the homozygous mutant (C/C) genotype was associated with an elevated risk of BC in South Indian women. A similar association was observed in a study conducted on mixed ethnicity (subjects were pooled from

| Genotype combination (XRCC2, XRCC3, RAD51) | BC Cases (N) | Controls (N) | OR (95% CI) | p-value |
|-------------------------------------------|-------------|-------------|-------------|---------|
| Arg/Arg, Thr/Thr, G/G                     | 185         | 203         | Reference   |         |
| Arg/His, Thr/Met, G/G                     | 27          | 19          | 1.56 (0.83–2.89) | 0.160  |
| Arg/His, Met/Met, G/G                     | 7           | 2           | 3.84 (0.78–18.72) | 0.096  |
| Arg/Arg, Thr/Thr, G/C                     | 50          | 65          | 0.84 (0.56–1.28) | 0.428  |
| Arg/Arg, Thr/Thr, C/C                     | 15          | 5           | 3.29 (1.17–9.23) | 0.024  |

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Fig 2. Protein-protein interaction. STRING software depicts the protein-protein interaction network of XRCC2, XRCC3 and RAD51. The first ten proteins that primarily interacts with XRCC2, XRCC3 and RAD51 is highlighted.

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19 studies including 13 countries) [44], Polish [40], and European [22] women. We also observed that the mutant CC genotype elevated the risk of metastasis in individuals with the homozygous mutant genotype. In line with our report, Weigmans et al. [46] speculated that breast tumors that overexpress RAD51 might have an elevated chance of disease progression and metastasis. Additionally, they observed that RAD51 promotes the expression of pro-metastatic genes and decreases the metastasis suppressor gene expression. Several meta-analyses highlighted the RAD51 G135C variant could function as a potential candidate biomarker for various cancers, particularly breast cancer [47–49]. Sekhar et al. [50] observed that the homozygous mutant variant (C/C) elevated BC risk in an ethnic-specific manner. However, on the other hand, few studies suggested that RAD51 tolerates very minimal dysfunctional sequence variation, and the RAD51 G135C variant might not contribute towards BC susceptibility [23, 26, 51]. In contrast, another study in Polish women highlighted that the RAD51 135C allele reduced the risk of BC in BRCA1 5382insC mutation carriers [52]. Furthermore, the identification of RAD51 foci in gBRCA mutant tumors was correlated with PARP inhibitor resistance [53, 54]. Overall, RAD51 could be therapeutically targeted in the future using small molecule inhibitors [55] and could be used as a marker and target in neoadjuvant endocrine treatment [56].

Up to our knowledge, the present study is the first to report the impact of genetic polymorphisms in crucial HRR gene on BC development in South Indian women. Future studies that investigate the mechanistic role of HRR gene polymorphisms on BC predisposition, and studies that evaluate the prognostic potential of these SNPs might enhance our current understanding of the precise role played by these variants towards mammary carcinogenesis. Investigating common genetic variants that predispose BC development in developing nations such as India might aid primary health care providers in formulating schemes that could enable early diagnosis and lessen disease burden.

Supporting information
S1 File. Genotyping results of the study participants.
(XLSX)

S1 Table. Association between XRCC2 Arg188His variant and BC clinicopathological characteristics.
(DOCX)

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References

1. Ponder BA, Antoniou A, Dunning A, Easton DF, Pharoah PD. Polygenic inherited predisposition to breast cancer. Cold Spring Harb Symp Quant Biol. 2005; 70:35–41. https://doi.org/10.1101/sqb.2005.70.029 PMID: 16869736.

2. Duncan L, Shen H, Gelaye B, Meijsen J, Ressler K, Feldman M, Peterson R, Domingue B. Analysis of polygenic risk score usage and performance in diverse human populations. Nat Commun. 2019; 10 (1):3328. https://doi.org/10.1038/s41467-019-11112-0 PMID: 31346163.

3. Kunnavil R, Thirthahalli C, Nooy S, Somanna S, Murthy N. Estimation of burden of female breast cancer in India for the year 2016, 2021 and 2026 using disability adjusted life years. Int J Comm Med Pub Health 2017; 3:1135–1140. https://doi.org/10.18203/2394-6040.ijcmph20161372.

4. Prakash R, Zhang Y, Feng W, Jasin M. Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins. Cold Spring Harb Perspect Biol. 2015; 7(4):a016600. https://doi.org/10.1101/cshperspect.a016600 PMID: 25833843.

5. Feltes BC. Architects meets Repairers: The interplay between homeobox genes and DNA repair. DNA Repair (Amst). 2019; 73:73–48. https://doi.org/10.1016/j.dnarep.2018.10.007 PMID: 30448208.

6. Bohlander SK, Kakadiya PM, Coysh A. Chromosome Rearrangements and Translocations, Editor(s): Boffetta Paolo, Hainaut Pierre, Encyclopedia of Cancer ( Third Edition). Academic Press, 2019;389–404, ISBN 9780128124857.

7. Morrical SW. DNA-pairing and annealing processes in homologous recombination and homology-directed repair. Cold Spring Harb Perspect Biol. 2015; 7(2):a016444. https://doi.org/10.1101/cshperspect.a016444 PMID: 25646379.

8. Zelensky A, Kanaar R, Wyman C. Mediators of homologous DNA pairing. Cold Spring Harb Perspect Biol. 2014; 6(12):a016451. https://doi.org/10.1101/cshperspect.a016451 PMID: 25301930.

9. Shin A, Lee KM, Ahn B, Park CG, Noh SK, Park DY, et al. Genotype-phenotype relationship between DNA repair gene genetic polymorphisms and DNA repair capacity. Asian Pac J Cancer Prev. 2008; 9 (3):501–5. PMID: 18990028.

10. Cartwright R, Tambini CE, Simpson PJ, Thacker J. The XRCC2 DNA repair gene from human and mouse encodes a novel member of the recA/RAD51 family. Nucleic Acids Res. 1998; 26(13):3084–9. https://doi.org/10.1093/nar/26.13.3084 PMID: 9628903.

11. Johnson RD, Liu N, Jasin M. Mammalian XRCC2 promotes the repair of DNA double-strand breaks by homologous recombination. Nature. 1999; 401(6751):397–9. https://doi.org/10.1038/43932 PMID: 10517641.

12. Tambini CE, Spink KG, Ross CJ, Hill MA, Thacker J. The importance of XRCC2 in RAD51-related DNA damage repair. DNA Repair (Amst). 2010; 9(5):517–25. https://doi.org/10.1016/j.dnarep.2010.01.016 PMID: 20189471.
13. Rafii S, O’Regan P, Xinarianos G, Azmy I, Stephenson T, Reed M, et al. A potential role for the XRCC2 R188H polymorphic site in DNA-damage repair and breast cancer. Hum Mol Genet. 2002; 11(12):1433–8. https://doi.org/10.1093/hmg/11.12.1433 PMID: 12023985.

14. Han J, Hankinson SE, Zhang SM, De Vivo I, Hunter DJ. Interaction between genetic variations in DNA repair genes and plasma folate on breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2004; 13(4):520–4. PMID: 15069914.

15. Brenneman MA, Wagener BM, Miller CA, Allen C, Nickoloff JA. XRCC3 controls the fidelity of homologous recombination: roles for XRCC3 in late stages of recombination. Mol Cell. 2002; 10(2):387–95. https://doi.org/10.1016/S1097-2765(02)00595-6 PMID: 12191483.

16. Matullo G, Guerrera S, Carturan S, Peluso M, Malaveille C, Davico L, et al. DNA repair gene polymorphisms, bulky DNA adducts in white blood cells and bladder cancer in a case-control study. Int J Cancer. 2001; 92(4):562–7. https://doi.org/10.1002/ijc.1228 PMID: 11306492.

17. Matullo G, Palli D, Peluso M, Guerrera S, Carturan S, Celentano E, et al. XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. Carcinogenesis. 2001; 22(9):1437–45. https://doi.org/10.1093/carcin/22.9.1437 PMID: 11532866.

18. Araujo FD, Pierce AJ, Stark JM, Jasmin S. Variant XRCC3 implicated in cancer is functional in homology-directed repair of double-strand breaks. Oncogene. 2002; 21(26):4176–80. https://doi.org/10.1038/sj.onc.1205539 PMID: 12037675.

19. Song YZ, Han FJ, Liu M, Xia CC, Shi WY, Dong LH. Association between Single Nucleotide Polymorphisms in XRCC3 and Radiation-Induced Adverse Effects on Normal Tissue: A Meta-Analysis. PloS One. 2015; 10(6):e0130388. https://doi.org/10.1371/journal.pone.0130388 PMID: 26091483.

20. Costa S, Pinto D, Pereira D, Rodrigues H, Cameselle-Teijeiro J, Medeiros R, Schmitt F. DNA repair polymorphisms might contribute differentially on familial and sporadic breast cancer susceptibility: a study on a Portuguese population. Breast Cancer Res Treat. 2007; 103(2):209–17. https://doi.org/10.1007/s10549-006-9364-z PMID: 17063276.

21. Dufloth RM, Costa S, Schmitt F, Zeferino LC. DNA repair gene polymorphisms and susceptibility to familial breast cancer in a group of patients from Campinas, Brazil. Genet Mol Res. 2005; 4(4):771–82. PMID: 16475125.

22. Grešner P, Jabłońska E, Gromadzińska J. Rad51 paralogs and the risk of unselected breast cancer: A case-control study. PLoS One. 2020; 15(1):e0226976. https://doi.org/10.1371/journal.pone.0226976 PMID: 31905201.

23. Tulbah S, Alabdulkarim H, Alanazi M, Parine NR, Shaik J, Pathan AA, et al. Polymorphisms in RAD51 and their relation with breast cancer in Saudi females. Onco Targets Ther. 2016; 9:269–77. https://doi.org/10.2147/OTT.S93343 PMID: 26834486.

24. Jara L, Dubois K, Gaete D, de Mayo T, Ratkevicius N, Bravo T, et al. Variants in DNA double-strand break repair genes and risk of familial breast cancer in a South American population. Breast Cancer Res Treat. 2010; 122(3):813–22. https://doi.org/10.1007/s10549-009-0709-z PMID: 20054644.

25. Thyagarajan B, Anderson KE, Folsom AR, Jacobs DR Jr, Lynch CF, Bargaje A, et al. No association between XRCC1 and XRCC3 gene polymorphisms and breast cancer risk: Iowa Women’s Health Study. Cancer Detect Prev. 2006; 30(4):313–21. https://doi.org/10.1016/j.cdp.2006.07.002 PMID: 16963196.

26. Brooks J, Shore RE, Zeleniuch-Jacquotte A, Currie D, Afanasyeva Y, Koenig KL, et al. Polymorphisms in RAD51, XRCC2, and XRCC3 are not related to breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2008; 17(4):1016–9. https://doi.org/10.1158/1055-9965.EPI-08-0065 PMID: 18398049.

27. Ritchie MD, Hahn LW, Rooldi N, Bailey LR, Dupont WD, Pari FF, Moore JH. Multifactor-dimensionality reduction reveals higher-order interactions among estrogen-metabolism genes in sporadic breast cancer. Am J Hum Genet. 2001; 69(1):138–47. https://doi.org/10.1086/321276 PMID: PMC1226028. PMID: 11404819.

28. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019; 47(D1):D607–D613. https://doi.org/10.1093/nar/gky1131 PMID: PMC623986. PMID: 30476243.

29. Thacker J. The RAD51 gene family, genetic instability and cancer. Cancer Lett. 2005; 219(2):125–35. https://doi.org/10.1016/j.canlet.2004.08.018 PMID: 15723711.

30. Qureshi Z, Mahjabeen I, Baig R, Kiyani M. Correlation between selected XRCC2, XRCC3 and RAD51 gene polymorphisms and primary breast cancer in women in Pakistan. Asian Pac J Cancer Prev. 2014; 15(23):10225–8. https://doi.org/10.7314/apjcp.2014.15.23.10225 PMID: 25556451.

31. Silva SN, Tomar M, Paulo C, Gomes BC, Azevedo AP, et al. Breast cancer risk and common single nucleotide polymorphisms in homologous recombination DNA repair pathway genes XRCC2, XRCC3, NBS1 and RAD51. Cancer Epidemiol. 2010; 34(1):85–92. https://doi.org/10.1016/j.canep.2009.11.002 PMID: 20004634.
32. Millikan RC, Player JS, Decotret AR, Tse CK, Keku T. Polymorphisms in DNA repair genes, medical exposure to ionizing radiation, and breast cancer risk. Cancer Epidemiol Biomarkers Prev. 2005; 14 (10):2326–34. https://doi.org/10.1158/1055-9965.EPI-05-0186 PMID: 16214912.

33. Yu KD, Chen AX, Qiu LX, Fan L, Yang C, Shao ZM. XRCC2 Arg188His polymorphism is not directly associated with breast cancer risk: evidence from 37,369 subjects. Breast Cancer Res Treat. 2010; 123 (1):219–25. https://doi.org/10.1007/s10549-010-0753-y PMID: 20127279.

34. He Y, Zhang Y, Jin C, Deng X, Wei M, Wu Q, et al. Impact of XRCC2 Arg188His polymorphism on cancer susceptibility: a meta-analysis. PLoS One. 2014; 9(3):e91202. https://doi.org/10.1371/journal.pone.0091202 PMID: 24621646.

35. Kong B, Lv ZD, Chen L, Shen RW, Jin LY, Yang ZC. Lack of an association between XRCC2 R188H polymorphisms and breast cancer: an update meta-analysis involving 35,422 subjects. Int J Clin Exp Med. 2015; 8(9):15808–14. PMID: PMC4658969. PMID: 26629080.

36. Dashi S, Taherian-Esfahani Z, Keshtkari A, Ghafouri-Fard S. Associations between XRCC3 Thr241Met polymorphisms and breast cancer risk: systematic-review and meta-analysis of 55 case-control studies. BMC Med Genet 2019; 20:79. https://doi.org/10.1186/s12881-019-0809-8 PMID: 31077150.

37. Mao CF, Qian WY, Wu JZ, Sun DW, Tang JH. Association between the XRCC3 Thr241Met polymorphism and breast cancer risk: an updated meta-analysis of 36 case-control studies. Asian Pac J Cancer Prev. 2014; 15(16):6613–8. https://doi.org/10.7314/apjcp.2014.15.16.6613 PMID: 25169497.

38. Sangrajrang S, Schmezer P, Burkholder I, Boffetta P, Brennan N, Woelfelschneider A, et al. The XRCC3 Thr241Met polymorphism and breast cancer risk: a case-control study in a Thai population. Bioinformatics. 2007; 23(12):i52–3. https://doi.org/10.1093/bioinformatics/btm246 PMID: 17701750.

39. Santos RA, Teixeira AC, Maynorayo MB, Carrara HH, Andrade JM, Takahashi CS. DNA repair genes XRCC1 and XRCC3 polymorphisms and their relationship with the level of micronuclei in breast cancer patients. Genet Mol Biol. 2010; 33(4):837–40. https://doi.org/10.1590/S1415-475720100005000082 PMID: PMC3036161. PMID: 21637570.

40. Romanowicz-Makowska H, Smolarz B, Zadrożny W, Westfal B, Baszczyński J, Polań I, Sporny S. Single nucleotide polymorphisms in the homologous recombination repair genes and breast cancer risk in Polish women. Tohoku J Exp Med. 2011; 224(3):201–8. https://doi.org/10.1620/tjem.224.201 PMID: 21701125.

41. Han J, Hankinson SE, Ranu H, De Vivo I, Hunter DJ. Polymorphisms in DNA double-strand break genes and breast cancer risk in the Nurses’ Health Study. Carcinogenesis. 2004; 25(2):189–95. https://doi.org/10.1093/carcin/bgh002 PMID: 14578164.

42. Devi KR, Ahmed J, Nair K, Mukherjee K, Majumdar G, Chenkual S, Zonunmawia JC. DNA Repair Mechanism Gene, XRCC1A (Arg194Trp) but not XRCC3 (Thr241Met) Polymorphism Increased the Risk of Breast Cancer in Premenopausal Females: A Case-Control Study in Northeastern Region of India. Technol Cancer Res Treat. 2017; 16(6):1150–1159. https://doi.org/10.1177/1533034617736162 PMID: PMCID: PMC5762082. PMID: 29332455.

43. Ramadan RA, Desouky LM, Elnaggar MA, Moaaz M, Elsherif AM. Association of DNA repair genes XRCC1 (Arg399Gln), (Arg194Trp) and XRCC3 (Thr241Met) polymorphisms with the risk of breast cancer: a case-control study in Egypt. Genet Test Mol Biomarkers. 2014; 18(11):754–60. https://doi.org/10.1089/gtmb.2014.0191 PMID: 25340946.

44. Antoniou AC, Sinilnikova OM, Simard J, Léoné M, Dumont M, Neuhausen SL, et al. RAD51 135G>C polymorphism modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. Am J Hum Genet. 2007; 81(6):1186–200. https://doi.org/10.1086/522611 PMID: PMCID: PMC2276351. PMID: 17999359.

45. Kadoury L, Kote-Jarai Z, Hubert A, Durocher F, Abeliovich D, Glaser B, et al. A single-nucleotide polymorphism in the RAD51 gene modifies breast cancer risk in BRCA2 carriers, but not in BRCA1 carriers or noncarriers. Br J Cancer. 2004; 90(10):2002–5. https://doi.org/10.1038/sj.bjc.6601837 PMID: PMCID: PMC2409456. PMID: 15138485.

46. Wiegmans AP, Al-Ejeh F, Chee N, Yap PY, Gorski JJ, Da Silva L, et al. Rad51 supports triple negative breast cancer metastasis. Oncotarget. 2014; 5(10):3261–72. https://doi.org/10.18632/oncotarget.1923 PMID: PMCID: PMC4102808. PMID: 24811120.

47. Zhao M, Chen P, Dong Y, Zhu X, Zhang X. Relationship between Rad51 G135C and G172T variants and the susceptibility to cancer: a meta-analysis involving 54 case-control studies. PLoS One. 2014; 9 (1):e87259. https://doi.org/10.1371/journal.pone.0087259 PMID: PMCID: PMC3903631. PMID: 24475258.

48. Wang W, Li JL, He XF, Li AP, Cai YL, Xu N, et al. Association between the RAD51 135 G>C polymorphism and risk of cancer: a meta-analysis of 19,068 cases and 22,630 controls. PLoS One. 2013; 8(9):e75153. https://doi.org/10.1371/journal.pone.0075153 PMID: PMCID: PMC3766794. PMID: 24040396.

49. Sun H, Bai J, Chen F, Jin Y, Yu Y, Jin L, Fu S. RAD51 G135C polymorphism is associated with breast cancer susceptibility: a meta-analysis involving 22,399 subjects. Breast Cancer Res Treat. 2011; 125 (1):157–61. https://doi.org/10.1007/s10549-010-0922-z PMID: 20454923.
50. Sekhar D, Pooja S, Kumar S, Rajender S. RAD51 135G>C substitution increases breast cancer risk in an ethnic-specific manner: a meta-analysis on 21,236 cases and 19,407 controls. Sci Rep. 2015; 5:11588. https://doi.org/10.1038/srep11588 PMCID: PMC4479800. PMID: 26108708

51. Le Calvez-Kelm F, Oliver J, Damiola F, Forey N, Robinot N, Durand G, et al. RAD51 and breast cancer susceptibility: no evidence for rare variant association in the Breast Cancer Family Registry study. PLoS One. 2012; 7(12):e52374. https://doi.org/10.1371/journal.pone.0052374 PMCID: PMC3531476. PMID: 23300655

52. Jakubowska A, Narod SA, Goldgar DE, Mierzejewski M, Masoń B, Nej K, et al. Breast cancer risk reduction associated with the RAD51 polymorphism among carriers of the BRCA1 5382insC mutation in Poland. Cancer Epidemiol Biomarkers Prev. 2003; 12(5):457–9. PMID: 12750242.

53. Wang Y, Bernhardy AJ, Cruz C, Krais JJ, Nacson J, Nicolas E, et al. The BRCA1-Δ11q Alternative Splice Isoform Bypasses Germline Mutations and Promotes Therapeutic Resistance to PARP Inhibition and Cisplatin. Cancer Res. 2016; 76(9):2778–90. https://doi.org/10.1158/0008-5472.CAN-16-0186 PMCID: PMC4874568. PMID: 27197267

54. Cruz C, Castroviejo-Bermejo M, Gutiérrez-Enríquez S, Llop-Guevara A, Ibrahim YH, Gris-Oliver A, et al. RAD51 foci as a functional biomarker of homologous recombination repair and PARP inhibitor resistance in germline BRCA-mutated breast cancer. Ann Oncol. 2018; 29(5):1203–1210. https://doi.org/10.1093/annonc/mdy099 PMCID: PMC5961353. PMID: 29635390

55. Huang F, Mazin AV. A small molecule inhibitor of human RAD51 potentiates breast cancer cell killing by therapeutic agents in mouse xenografts. PLoS One. 2014; 9(6):e100993. https://doi.org/10.1371/journal.pone.0100993 PMCID: PMC4074124. PMID: 24971740

56. Jia Y, Song Y, Dong G, Hao C, Zhao W, Li S, et al. Aberrant Regulation of RAD51 Promotes Resistance of Neoadjuvant Endocrine Therapy in ER-positive Breast Cancer. Sci Rep. 2019; 9:12939. https://doi.org/10.1038/s41598-019-49373-w PMID: 31506496