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ORIGINAL ARTICLE

Association between *RAD51*, *XRCC2* and *XRCC3* gene polymorphisms and risk of ovarian cancer: a case control and an *in silico* study

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Abstract

Homologous recombination (HR) is one of the important mechanisms in repairing double-strand breaks to maintain genomic integrity and DNA stability from the cytotoxic effects and mutations. Various studies have reported that single nucleotide polymorphisms (SNPs) in the HR-associated genes may have a significant association with ovarian cancer (OCa) risk but the results were inconclusive. In the present study, five polymorphisms of HR-associated genes (*RAD51*, *XRCC2* and *XRCC3*) were genotyped by allelic discrimination assay in 200 OCa cases and 200 healthy individuals. The association with OCa risk was evaluated by unconditional logistic regression analyses. The results revealed that the mutant allele in both rs1801320 (CC) and rs1801321 (TT) of *RAD51* gene was associated with increased risk of OCa (odds ratio [OR] 3.79, 95% confidence interval [CI] 1.21–11.78, p = 0.014 and OR 1.61, 95% CI 1.06–2.45, p = 0.025, respectively). Moreover, a significant association of TT allele (OR 4.68, 95% CI 1.27–17.15, p = 0.011) of rs3218536 of *XRCC2* gene with OCa was observed. Stratified analysis results showed that patients with early menarche and stages 3 and 4 were found to be associated with rs1801321 of *RAD51 gene* and rs1799794 of *XRCC3* gene. *In silico* analysis predicted that the two missense SNPs (rs3218536 and rs1799794) were found to have an impact on the protein structure, stability and function. The present study suggested that *RAD51* and *XRCC2* gene polymorphisms might have an impact on the OCa risk in the South Indian population. However, studies with a larger sample and on different populations are needed to support the conclusions.

Keywords DNA damage · DNA repair · Double-strand breaks · Homologous recombination · *RAD51* · Ovarian neoplasms

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Introduction

DNA double-strand breaks (DSBs) are the most lethal type of damage, which must be repaired efficiently to maintain genomic integrity and genetic alterations, which can otherwise lead to cancer [1]. DNA damaging agents such as endogenous and exogenous elements affect DNA stability, which results in DSBs during the S phase [2]. Most of the cell deaths and malignancies are initiated by the accumulation of these unrepaired DSBs. These DSBs are repaired by several mechanisms in which the homologous recombination (HR) is considered a key pathway [3]. However, HR is restricted to the S and G2 phases by cell cycle [4]. In the past few decades, HR has strong consideration and immense progress has been achieved in molecular specifics and regulation of this pathway [1]. Defects in the HR repair pathway have been reported to be closely related to various malignancies in humans [5]. The candidate genes *RAD51*, *XRCC2,* and *XRCC3* are found to be attractive and serve as essential proteins during the HR

process [6–8]. The *RAD51* gene complex plays a critical role in DSB repair by HR along with its paralogs such as *XRCC2* and *XRCC3* genes by chromosome segregation and apoptotic response to DSBs [9, 10]. The cells that are deficient in these gene products eventually result in defective HR and exhibits genomic stability [11, 12].

Over the decade, studies have reported that single nucleotide polymorphisms (SNPs) in the DNA repair pathway may influence the susceptibility to cancer by modifying repair capability [13]. In the *RAD51* gene, the polymorphisms GC135 (rs1801320) and GT172 (rs1801321) modify the *Rad51* protein by increasing the predisposition to cancer due to a reduction in DNA damage repairing capacity [11]. The *XRCC2* R188H (rs3218536) polymorphism is shown to have severe homozygous mutations that may be associated with ovarian cancer (OCa) risk [14, 15]. Furthermore, the polymorphisms Thr241Met (rs861539) and rs1799794 of the *XRCC3* gene were widely studied in various cancer progressions [16–18]. These functional polymorphisms are potential susceptibility loci, recent studies showed that the association of these polymorphisms with various cancers but the results were debatable [19, 20]. The functions of the selected polymorphisms for this study are represented in Supplementary Table S1. The genetic variants in these genes have been identified as a potential risk for cancer progression such as breast, pancreatic, liver, lungs, and ovary; inconclusive results were reported [20–26]. However, inadequate studies are available related to these genetic polymorphisms and their association with OCa risk and there are no studies available concerning the south Indian population. The present is the first study conducted on the South Indian population.

Additionally, *in silico* approach was applied to predict the impact of the missense SNPs (rs3218536 and rs861539) on the protein structure, function and stability and to understand the pathogenicity and disease association of the SNPs. The genetic defects in *RAD51*, *XRCC2,* and *XRCC3* genes can affect the repairing efficiency since the genes are highly polymorphic, which could lead to the progression of OCa. The outcome of the study may contribute to early diagnosis and may increase the benefit in women with OCa by altering the therapy. Based on these interpretations, we examined the role of polymorphisms in the DNA repair pathway (HR) genes and their susceptibility to OCa risk in South Indian women.

Materials and methods

Study subjects

Blood samples were collected from 200 histologically proven OCa patients treated at the Department of Oncology, Sri Ramachandra Medical Center (Chennai, India) between the years 2016 and 2019. Control samples consisting of age-matched 200 healthy volunteers with no prior cancer history were recruited for this study. Clinical characteristics of the cases and controls are given in Table 1. The study was approved by the institutional ethical committee (Reference Number-IEC-NI/17/JUN/60/80) and written informed consent was obtained from each participant.

DNA extraction

Peripheral blood samples (2 mL) were collected using ethylenediaminetetraacetic acid (EDTA) vacutainers (BD, NJ,

Table 1 Clinical characteristics of ovarian cancer patients (n = 200) and controls (n = 200)

Characteristics	Number of cases (%)	Number of controls (%)	p-value
Age (years)			
Median	51	51	0.43*
Range	20–80	20–80	
BMI			
≤ 24	103 (52.0)	77 (38.5)	< 0.05*
> 24	97 (48.0)	123 (61.5)	
Menarche			
≤ 13 years old	103 (51.5)	101 (50.5)	
> 13 years old	97 (48.5)	99 (49.5)	0.471**
Menopausal status			
Pre-menopausal	35 (17.5)	57 (28.5)	
Post-menopausal	165 (82.5)	143 (71.5)	< 0.05**
FIGO stage			
I	17 (8.5)	–	–
II	13 (6.5)	–	–
III	154 (77.0)	–	–
IV	16 (8.0)	–	–
Histological subtypes			
Serous	60 (30.0)	–	–
Mucinous	17 (8.5)	–	–
Endometrioid	49 (24.5)	–	–
Clear-cell	20 (10.0)	–	–
Undifferentiated	40 (20.0)	–	–
Others	14 (7.0)	–	–
Histological grade (G)			
1	80 (40.0)	–	–
2	108 (54.0)	–	–
3	12 (6.0)	–	–

BMI body mass index; *FIGO* the International Federation of Gynecology and Obstetrics; *G* grade

*Student t-test 2 tailed p-value

**Chi-square test; n, number of samples

USA) from all the study participants. Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) by following the manufacturer's protocol. The quantity and quality of the isolated DNA samples were checked by 0.8% agarose gel electrophoresis and nano spectrophotometer (Thermo Fisher Scientific/NanoDrop Products, Wilmington, DE, USA), the DNA samples were then stored at − 20 °C for further analysis.

Genotyping

In the present study, five SNPs of three DNA repair genes were selected which are involved in various intracellular processes and have a role in genomic integrity maintenance. All the SNPs of these selected genes were selected from the previous literature (Supplementary Table S1). TaqMan allelic discrimination assay was performed using predesigned probes for genotyping the polymorphisms rs1801320, rs1801321, rs3218536, rs1799794, and rs861539. All the polymorphisms were genotyped by a 7900HT fast real-time PCR system (Applied Biosystems, Foster City, CA, USA) using TaqMan SNP genotyping assays (Thermo Scientific, Waltham, MA, USA). The details of the SNP genotyping assays are represented in Supplementary Table S1. All the reactions were carried out in a 384-well array according to the manufacturer's instructions in a 5 µL reaction containing 1 µL of genomic DNA, 0.25 µL of 40X SNP genotyping assay mix, and 3.75 µL of 2X universal TaqMan master mix (Applied Biosystems). The annealing temperature for the amplification was 60 °C. The genotypes were called up automatically using the TaqMan Genotyper software (Applied Biosystems, Foster City, CA, USA). The overall success rate for the genotyping assays was 99%. For quality control, random samples were genotyped in duplicate and had identical genotyping assignments.

Statistical analysis

Statistical analysis was performed using IBM SPSS v25 (SPSS Inc., Chicago, IL). χ^2 was used to calculate the expected genotype and allele frequencies for the observed polymorphisms in cases and controls and tested for Hardy Weinberg equilibrium. The chi-squared test was used to analyze the difference in genotypes and allele frequencies between OCa patients and the control group calculated using odds ratios (ORs) and 95% confidence intervals (CIs). SNPs were further investigated with three logistic regression models (additive, dominant and recessive) by ORs and 95% CIs. A p-value of less than 0.05 was considered statistically significant. HapMap data was used to compare the study results with other ethnicities.

In-silico analyses

In the present study, two missense polymorphisms, rs3218536 of *XRCC2* and rs861539 of *XRCC3* genes, were found to be located on the coding regions. Computational analysis tools were used to understand the functional effect of these polymorphisms on protein. The amino acid sequence was obtained for *XRCC2* and *XRCC3* genes from the UniProt and NCBI databases and the structure was predicted using SWISS-MODEL and Phyre2 [27, 28]. The functional impact of R188H and T241M on the protein was predicted using the seven online tools such as SIFT, PolyPhen-2, PROVEAN, SNAP2, PANTHER, Mutation Assessor, and fathmm [29–35]. Disease association and pathogenicity were predicted using MutPred, PhD-SNP, SNPs&GO, PON-P, Meta SNP and Dr.Cancer [36–41]. For structural analysis, the I-Mutant 3.0 and MUpro were used to predict protein stability after the amino acid substitution [42, 43]. Phylogenetic conservation was analyzed using ConSurf [44]. A self-optimized method (SOPMA) was used to predict the secondary structure of amino acids (alpha-helix, beta-turn and coil) in the whole database [45]. STRING v11.0 network analysis tool was used to predict the protein–protein network association [46]. The wild-type and mutant amino acids were located on native proteins and structure prediction was done using PyMOL v2.4 [47].

Results

Prediction of protein–protein interaction

Protein–protein interaction prediction analyses were performed using STRING v11.0. The results revealed that the *RAD51* protein to have an interaction with various proteins including *BRCA1*/2; *XRCC2* protein had an interaction with *BRCA1*/2, *RAD51* paralogs and other proteins and *XRCC3* protein interacted with *RAD51*, *XRCC2*, *BRCA2* and other proteins (Fig. 1). Thus, revealing that the genes selected are in strong interaction with other vital proteins in the HR pathway reinforcing the eminent role of the selected genes in the DNA DSB repair.

Case control study

The present study has 400 subjects including 200 patients with clinical and histological confirmation of OCa and 200 healthy volunteers without any history of cancer as control were called up to examine the association between the polymorphisms rs1801320 and rs1801321 in the *RAD51* gene, rs3218536 in *XRCC2,* and rs1799794 and rs861539 in the *XRCC3* gene. As shown in Table 1, age in both cases and controls ranged from 20 to 80 years with a median of

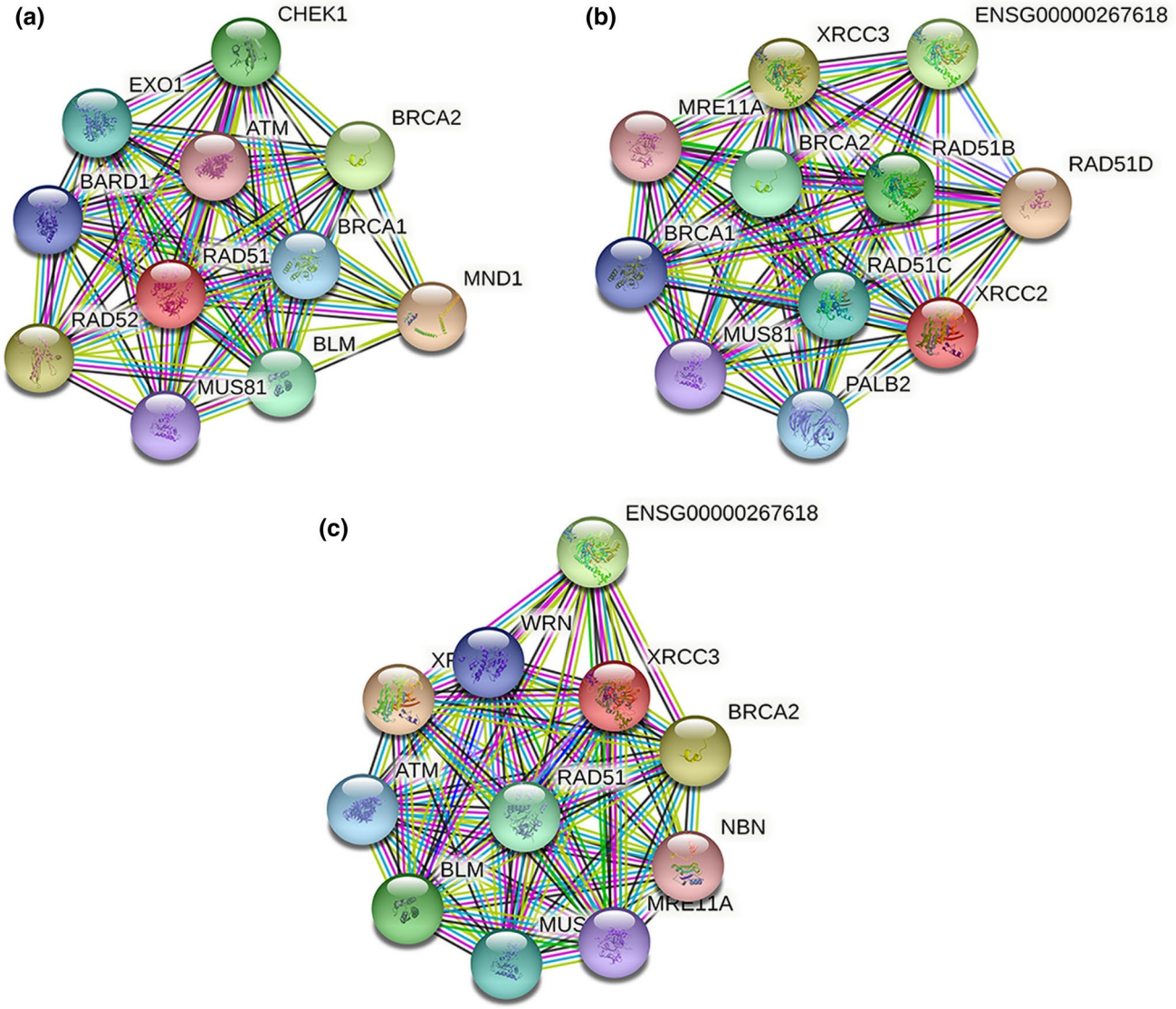

Fig. 1 The protein–protein interaction of *RAD51*, *XRCC2* and *XRCC3* genes were analysed using the String network analysis tool

51 years. The Student's t-test revealed a significant association between the cases and controls on body mass index (BMI) with a two-tailed p-value < 0.05. The chi-square test has shown a significant association between pre- and post-menopausal cases and controls with the two-tailed p-value < 0.05. Among the OCa patients (n = 200), 30% were serous, 24.5% were endometrioid, 20% were undifferentiated, 10% were clear cell, 8.5% were mucinous and 14% were others. Seventy-seven percent of the OCa patients were diagnosed at stage 3. G2 grade accounted for 54% of the cases.

Distribution of *RAD51*, *XRCC2*, and *XRCC3* gene polymorphisms on ovarian cancer

The allele distributions of *RAD51*, *XRCC2,* and *XRCC3* gene polymorphisms were compared between the present study and other populations using HapMap data and are represented in Fig. 2. The results revealed that the variant allele of the *XRCC2* rs3218536 polymorphism was found to be higher in the South Indian population compared to other populations. The distribution of the genotypes and allele frequency obtained from all the studied SNPs is presented

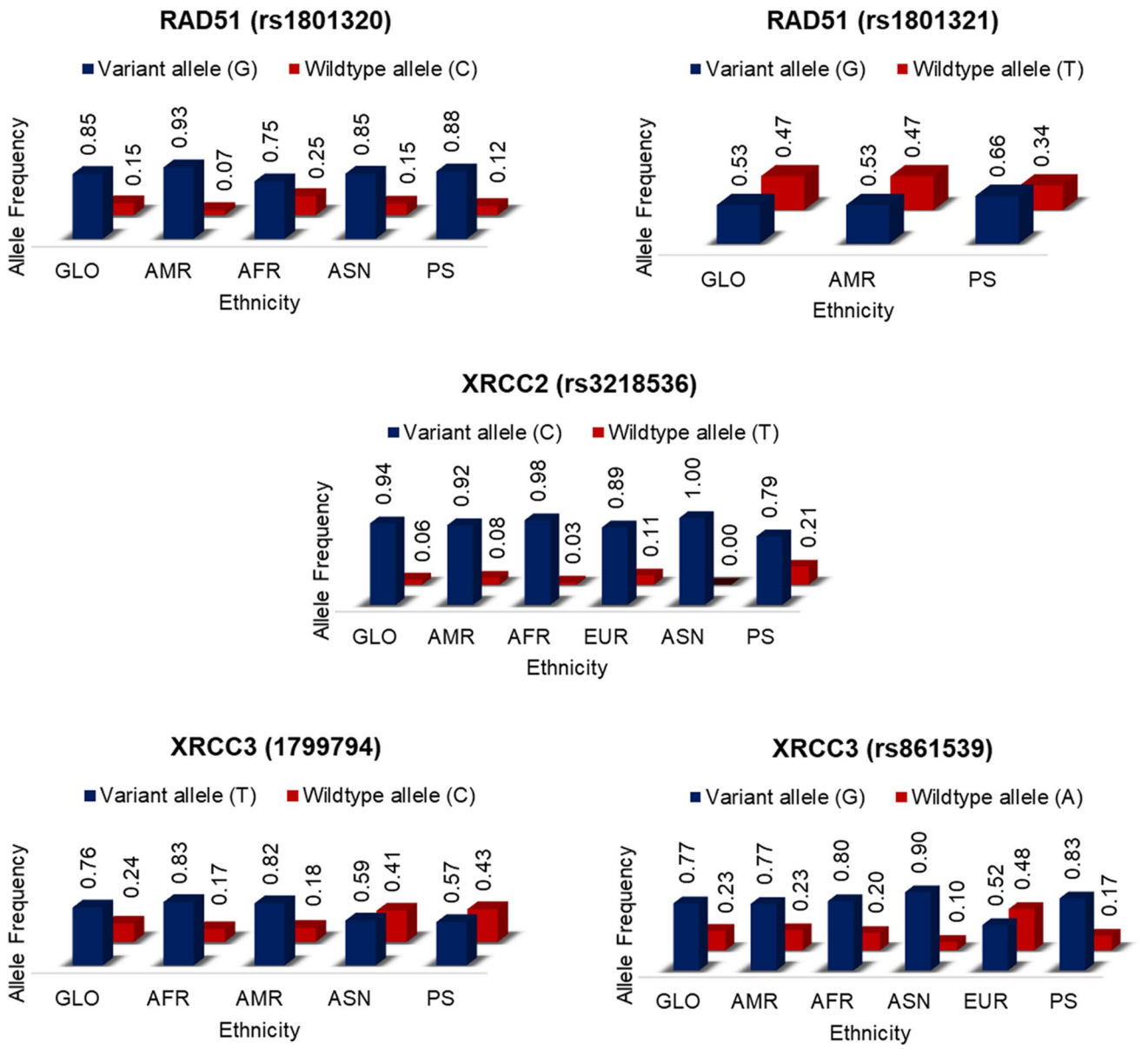

Fig. 2 Comparison of HapMap data for allele frequency of the present study with other ethnicities. *GLO*, Global; *AMR*, American; *AFR*, African; *ASN*, Asian; *EUR*, European; *PS*, Present Study

in Table 2. The results revealed a significant association between *RAD51* (rs1801320 and rs1801321) and *XRCC2* (rs3218536) gene polymorphisms and OCa risk. Among the five polymorphisms studied, in rs1801320 of *RAD51*, the frequency of a variant allele (CC) was observed to be significantly higher in OCa patients when compared to controls (OR 3.79, 95% CI 1.21–11.78, p = 0.014). Similarly, the C allele frequency of respective polymorphism was also found to be significantly higher in cases when compared with controls (OR 1.55, 95% CI 1.04–2.31, p = 0.028) which reveals an increased risk of OCa. For polymorphism, rs1801321, the TT genotype (OR 1.61, 95% CI 1.06–2.45, p = 0.025) and T allele frequency (OR 1.52, 95% CI 1.14–2.03, p = 0.003) revealed a significant association with OCa risk.

For the polymorphism rs3218536 of the *XRCC2* gene the heterozygous allele (CT) was found to be higher in cases when compared to controls (OR 2.23, 95% CI 1.39–3.58, p = < 0.001) and in the same way homozygous mutant allele (TT) was found to be increased in cases than controls (OR 4.68, 95% CI 1.27–17.15, p = 0.011). Similarly, the T allele frequency of respective polymorphism was found to be higher in cases compared to controls (OR 0.43, 95% CI 0.29–0.64, p = < 0.001). Thus, the polymorphism rs3218536 of the *XRCC2* gene revealed a strong significant association

Table 2 Distribution of five selected SNPs in *RAD51*, *XRCC2* and *XRCC3* gene in ovarian cancer cases

Polymorphisms	Genotypes	Cases (n = 200) (%)	Controls (n = 200) (%)	OR (95% CI)	p-value*
RAD51 rs1801320	GG	144 (72.0)	156 (78.0)	Ref	
	GC	42 (21.0)	40 (20.0)	1.13 (0.69–1.85)	0.605
	CC	14 (7.0)	4 (2.0)	3.79 (1.21–11.78)	0.014
	G allele frequency	330 (82.5)	352 (88.0)	Ref	
	C allele frequency	70 (17.5)	48 (12.0)	1.55 (1.04–2.31)	0.028
RAD51 rs1801321	GG	109 (54.5)	124 (62.0)	Ref	
	GT	6 (3.0)	16 (8.0)	0.42 (0.16–1.12)	0.079
	TT	85 (42.5)	60 (30.0)	1.61 (1.06–2.45)	0.025
	G allele frequency	224 (56.0)	264 (66.0)	Ref	
	T allele frequency	176 (44.0)	136 (34.0)	1.52 (1.14–2.03)	0.003
XRCC2 rs3218536	CC	126 (63.0)	161 (80.5)	Ref	
	CT	63 (31.5)	36 (18.0)	2.23 (1.39–3.58)	< 0.001
	TT	11 (5.5)	3 (1.5)	4.68 (1.27–17.15)	0.011
	C allele frequency	315 (78.8)	358 (82.5)	Ref	
	T allele frequency	85 (21.3)	42 (17.5)	2.30 (1.54–3.42)	< 0.001
XRCC3 rs1799794	TT	70 (35.0)	65 (32.5)	Ref	
	TC	93 (46.5)	97 (48.5)	0.89 (0.57–1.38)	0.606
	CC	37 (18.5)	38 (19.0)	0.90 (0.51–1.59)	0.727
	T allele frequency	233 (58.3)	227 (56.8)	Ref	
	C allele frequency	167 (41.8)	173 (43.3)	0.94 (0.71–1.24)	0.668
XRCC3 rs861539	GG	136 (68.0)	140 (70.0)	Ref	
	GA	49 (24.5)	51 (25.5)	0.98 (0.62–1.56)	0.962
	AA	15 (7.5)	9 (4.5)	1.71 (0.72–4.05)	0.214
	G allele frequency	321 (80.3)	331 (82.8)	Ref	
	A allele frequency	79 (19.8)	69 (17.3)	1.18 (0.82–1.68)	0.362

n Number of samples; *Ref* Reference; *OR* odds ratio; *CI* confidence interval

*Chi-square (χ^2); p-value ≤ 0.05 considered as statistically significant

with OCa risk. Furthermore, the results suggested that the genotypes of *XRCC3* gene polymorphisms are not statistically significant with OCa risk.

Distribution of genotypes on three genetic models

Disease susceptibility loci depending on the genetic models were tested by statistical power in genetic association studies. Thus, the additive, dominant and recessive genetic models were analyzed to check the genotype frequencies in OCa and are given in Table 3. A significant association between rs1801320 polymorphism of *RAD51* gene was observed in recessive (CC vs GG + GC) and additive (C vs G) models (OR 0.27, 95% CI 0.08–0.83, p = 0.016 and OR 1.55, 95% CI 1.04–2.31, p = 0.028**,** respectively). For the rs1801321 polymorphism, the recessive (TT vs GG + GT) OR 0.57, 95% CI 0.38–0.87, p = 0.009 and additive (T vs G) (OR 1.52, 95% CI 01.14–2.03, p = 0.003) models revealed a significant association with OCa risk. The dominant (CC vs CT + TT), recessive (TT vs CC + CT) and additive (T vs C) models of *XRCC2* gene polymorphism rs3218536 revealed a significant association with OCa risk (OR 2.42, 95% CI 1.54–3.81, p = < 0.001, OR 0.26, 95% CI 0.07–0.95, p = 0.029 and OR 2.30, 95% CI 1.54–3.42, p = < 0.001**,** respectively). The genetic models of *XRCC3* polymorphisms rs1799794 and rs861539 did not reveal any association with OCa risk in the South Indian population.

Distribution of *RAD51*, *XRCC2*, and *XRCC3* polymorphism genotypes on FIGO stages, age at menarche, and subgroups among ovarian cancer patients

The *RAD51, XRCC2, and XRCC3* gene polymorphisms were studied among OCa patients with clinical characteristics on six clinicopathological parameters such as the International Federation of Obstetrics and Gynecology (FIGO) stages, grades, BMI, age at menarche, and menopausal status to understand the etiology of OCa. As shown in Table 4 the genotype frequencies of *RAD51*, *XRCC2,* and *XRCC3* gene polymorphisms were compared with FIGO stages in OCa. The results showed that the genotype GT (OR 10.11, 95% CI

Table 3 Analysis of the five selected SNPs based on three genetic models

Polymorphisms	Models	Genotypes	OR (95% CI)	p-Value*
RAD51 rs1801320	Dominant	GG vs GC + CC	1.37 (0.87–2.17)	0.166
	Recessive	CC vs GG + GC	0.27 (0.08–0.83)	0.016
	Additive	C vs G	1.55 (1.04–2.31)	0.028
RAD51 rs1801321	Dominant	GG vs GT + TT	1.36 (0.91–2.02)	0.128
	Recessive	TT vs GG + GT	0.57 (0.38–0.87)	0.009
	Additive	T vs G	1.52 (1.14–2.03)	0.003
XRCC2 rs3218536	Dominant	CC vs CT + TT	2.42 (1.54–3.81)	< 0.001
	Recessive	TT vs CC + CT	0.26 (0.07–0.95)	0.029
	Additive	T vs C	2.30 (1.54–3.42)	< 0.001
XRCC3 rs1799794	Dominant	TT vs TC + CC	0.89 (0.59–1.35)	0.597
	Recessive	CC vs TT + TC	1.03 (0.62–1.70)	0.898
	Additive	C vs T	0.94 (0.71–1.24)	0.668
XRCC3 rs861539	Dominant	GG vs GA + AA	1.09 (0.71–1.67)	0.665
	Recessive	AA vs GG + GA	0.58 (0.24–1.36)	0.207
	Additive	A vs G	1.18 (0.82–1.68)	0.362

OR odds ratio; *CI* confidence interval

*Chi-square (χ^2); p-value ≤ 0.05 considered as statistically significant

3.35–30.45, p = < 0.001) is found to be higher in stage I and II OCa patients and TT (OR 0, 95% CI 0–0, p = < 0.001) and T (OR 0.30, 95% CI 0.15–0.60, p = < 0.001) allele frequency was found to be higher in stage III and IV OCa patients for *RAD51* rs1801321 gene polymorphism. In rs1799794 *XRCC3* the C allele frequency (OR 1.73, 95% CI 0.99–3.01, p = 0.048) was found to be higher in stage III and IV OCa patients. Furthermore, the *RAD51* rs1801321 gene polymorphism genotype TT (OR 0.42, 95% CI 0.24–0.76, p = 0.004) and T allele frequency (OR 0.44, 95% CI 0.29–0.66, p = < 0.001) was found to be higher in OCa patients with menarche age above 13 (Table 4). The other factors such as BMI and menopausal status did not reveal any association with OCa risk on the studied population.

The polymorphisms were further studied with different histological subtypes of OCa with controls as presented in Supplementary Table S2. The results showed that the *RAD51* (rs1801320 and rs1801321) and *XRCC2* (rs3218536) gene polymorphisms are in significant association with histological subtypes of OCa. For rs1801320, genotype CC (p = < 0.001) and G (p = 0.05) allele revealed a significant association with undifferentiated subtype and the CC (p = 0.020) genotype was found to be higher in other subtypes of OCa. The *RAD51* rs1801321 revealed a significant association between serous (GT, p = 0.031), mucinous (T, p = 0.026), endometrioid (TT, p = 0.019 and T, p = 0.001) and undifferentiated (T, p = 0.021) subtypes of OCa and controls. For *XRCC2* rs3218536, the mucinous (CT, p = 0.042; TT, p = 0.001 and T, p = 0.001), endometrioid (CT, p = 0.022 and T, p = 0.032), clear cell (CT, p = 0.013 and T, p = 0.006), undifferentiated (CT, p = < 0.001; TT, p = 0.002 and T, p = < 0.001) and others (TT, p = 0.001 and T, p = 0.019) OCa subtypes showed a significant association. Therefore, the results exposed that the *RAD51* and *XRCC2* gene polymorphisms are increasing the risk of OCa with histological subtypes. Furthermore, The *XRCC3* gene polymorphisms rs1799794 and rs861539 did not show association with any of the histological subtypes of OCa.

Functional analysis by *in-silico* tools

The functional analysis by *in-silico* tools revealed an impact of the polymorphisms on the protein structure and stability and its pathogenicity (Supplementary Table S3).

For the functional analysis SIFT, PolyPhen2, PROVEAN, SNAP2, PANTHER, Mutation Assessor and fathmm tools were used. The SIFT results revealed a tolerated effect for both the SNPs with a score of 0.98 (rs3218536) and 1.00 (rs861539). The PolyPhen2 results revealed that it was benign for rs3218536 (0.047) and possibly damaging (0.541) for rs861539. PROVEAN showed a neutral effect for both polymorphisms. The effect was predicted by the SNAP2 for both the SNPs. PANTHER tool classified both the SNPs as probably benign. The Mutation Assessor tool revealed a neutral effect for rs3218536 and a low effect for rs861539 SNPs. Passenger effect was observed for both SNPs by using the fathmm tool.

For the prediction of pathogenicity and disease association the MutPred2, PhD-SNP, SNPs&GO, PON-P, Meta SNP and Dr.Cancer tools were used. The MutPred2 results showed a non-pathogenic effect with a score of 0.135 for rs3218536 and 0.078 for rs861539 SNPs. The tools PhD-SNP, SNPs&GO, PON-P and Meta SNP revealed a neutral effect for both the SNPs. Dr. Cancer tool predicted that the

Table 4 Genotype and allele frequency distribution of studied gene polymorphisms on age at menarche and FIGO stages of the ovarian cancer patients

Odds ratios (95% CIs)		
Polymorphisms	Age at menarche	FIGO stages
	≤13 vs >13	Stage I,II vs III,IV
RAD51 rs1801320		
GG	Ref	Ref
GC	0.71 (0.36–1.43)	1.17 (0.46–2.98)
CC	0.87 (0.29–2.60)	0.97 (0.20–4.57)
G	Ref	Ref
C	0.80 (0.48–1.35)	1.06 (0.52–2.17)
RAD51 rs1801321		
GG	Ref	Ref
GT	3.38 (0.38–29.96)	10.11 (3.35–30.45)‡
TT	0.42 (0.24–0.76)†	0‡
G	Ref	Ref
T	0.44 (0.29–0.66)‡	0.30 (0.15–0.60)‡
XRCC2 rs3218536		
CC	Ref	Ref
CT	1.72 (0.93–3.19)	0.72 (0.30–1.74)
TT	1.36 (0.39–4.69)	0.50 (0.06–4.11)
C	Ref	Ref
T	1.45 (0.89–2.36)	0.70 (0.34–1.46)
XRCC3 rs1799794		
TT	Ref	Ref
TC	0.59 (0.32–1.12)	2.21 (0.81–5.99)
CC	1.46 (0.64–3.34)	2.94 (0.93–9.25)
T	Ref	Ref
C	1.08 (0.72–1.61)	1.73 (0.99–3.01)†
XRCC3 rs861539		
GG	Ref	Ref
GA	0.96 (0.49–1.84)	1.13 (0.46–2.76)
AA	2.75 (0.83–9.06)	0.89 (0.18–4.25)
G	Ref	Ref
A	1.40 (0.85–2.30)	1.01 (0.51–2.02)

n number of samples; *CI* confidence interval

†$p<0.05$; ‡$p<0.001$

rs3218536 causes disease and neutral effect for the rs861539 SNP.

For the prediction of protein stability, I-Mutant 3.0 and MUpro tools were used. I-Mutant 3.0 predicted decreased effect for rs3218536 (ΔΔG value = −1.04) and increased for rs861539 (ΔΔG value = 0.24). MUpro revealed a decrease in the stability of the protein with ΔΔG values of −1.127 and −0.340 for the SNPs rs3218536 and rs861539 respectively. Thus, based on the results from the various tools it was predicted that the SNPs rs3218536 of *XRCC2* and rs861539 of the *XRCC3* gene were found to have an impact on the protein function, stability and pathogenicity.

For the analysis of phylogenetic conservation, the ConSurf tool was used. The results revealed that 'R' residue of *XRCC2* protein was predicted to be a buried residue and conserved with a scale of 6 and 'T' residue of *XRCC3* protein was predicted to be an exposed variable with a scale of 3 (Fig. 3).

The SOPMA was used to predict the secondary structure of *XRCC2* and *XRCC3* to understand the beta-sheets and alpha-helix and coil distributions. For *XRCC2*, the result indicated alpha helix (111, 39.64%), extended Strand (53, 18.93%), beta turn (11, 3.93%) and random coil (105, 37.50%). For the *XRCC3*, the result showed alpha helix (160, 46.24%), extended strand (43, 12.43%), beta turn (8, 2.31%) and random coil (135, 39.02%). The R amino acid residue of *XRCC2* was located on the random coil and the T residue of *XRCC3* was located on the alpha helix (Fig. 4 [1a and 1b]). The wild-type and mutant amino acid position were located on the crystal structure of native *XRCC2* and *XRCC3* protein using PyMOL software and the image is represented in Fig. 4 (2a and 2b).

Discussion

The alteration of genetic variants in DNA repair pathway genes is well proven to be influencing an individual's capacity towards developing various cancers [26, 48–50]. Previous studies have shown that genes associated with DNA repair and in maintaining genome integrity play an important role in mutation prevention. The DNA DSBs are considered as the most toxic lesion and repaired by HR. *RAD51* gene and its paralogs *RAD51B*, *XRCC2,* and *XRCC3* genes promote the repair process by catalyzing the homology search and DNA strand invasion that represents HR repair [51–53]. Although SNPs in these genes have been documented to be either increasing predisposition or increased resistance, the influence of specific variations on repair phenotype and cancer risk has not been well-known [54, 55] since these genes play a significant role in the repair of DSBs caused by exogenous agents and endogenous metabolic products [56]. This was the first study emphasized to examine the role of polymorphisms in these candidate genes and their association with OCa in the South Indian population.

In the present case–control study, the five SNPs of three genes were genotyped for the association with OCa risk. The STRING network analysis showed that the *RAD51*, *XRCC2,* and *XRCC3* had a strong interaction with each other and with other important proteins within the HR network. Among the five polymorphisms studied, two were from promotor region 135G > C (rs1801320) and 172G > T (rs1801321) of the *RAD51* gene; Arg188His (rs3218536) from exon region of *XRCC2* gene; and A4541G (rs1799794) intron region and Thr241Met (rs861539) from exon region of *XRCC3* gene.

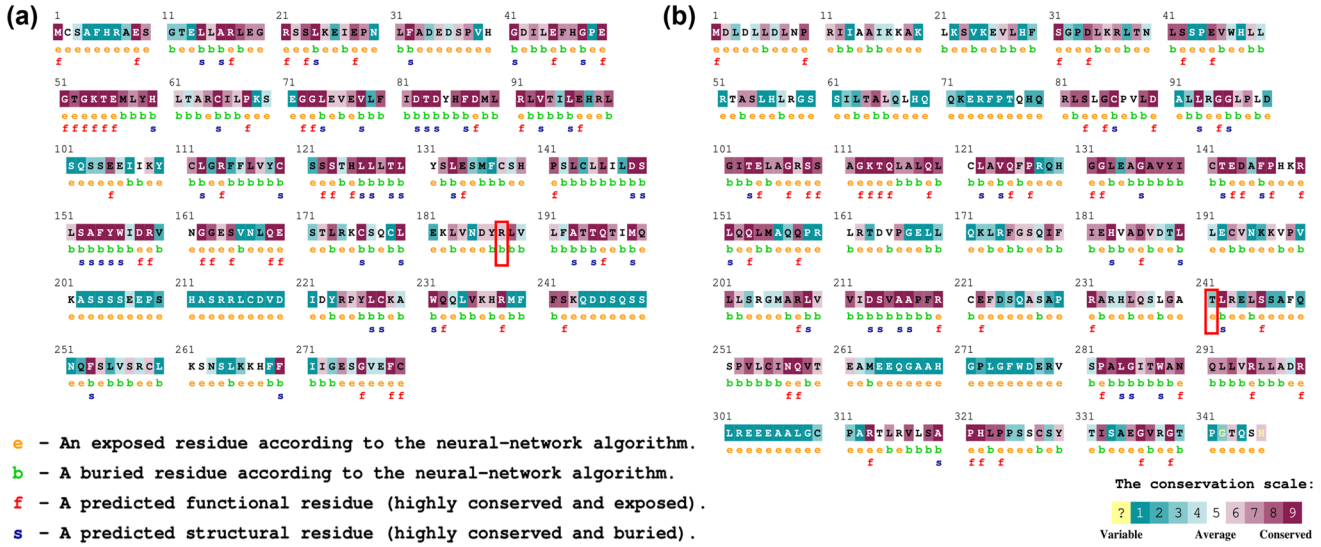

Fig. 3 Analysis of evolutionarily conserved amino acid residues of *XRCC2* and *XRCC3* by ConSurf. **a** 'R' residue at 188 position shows conserved. **b** 'T' residue at position 241 shows variables

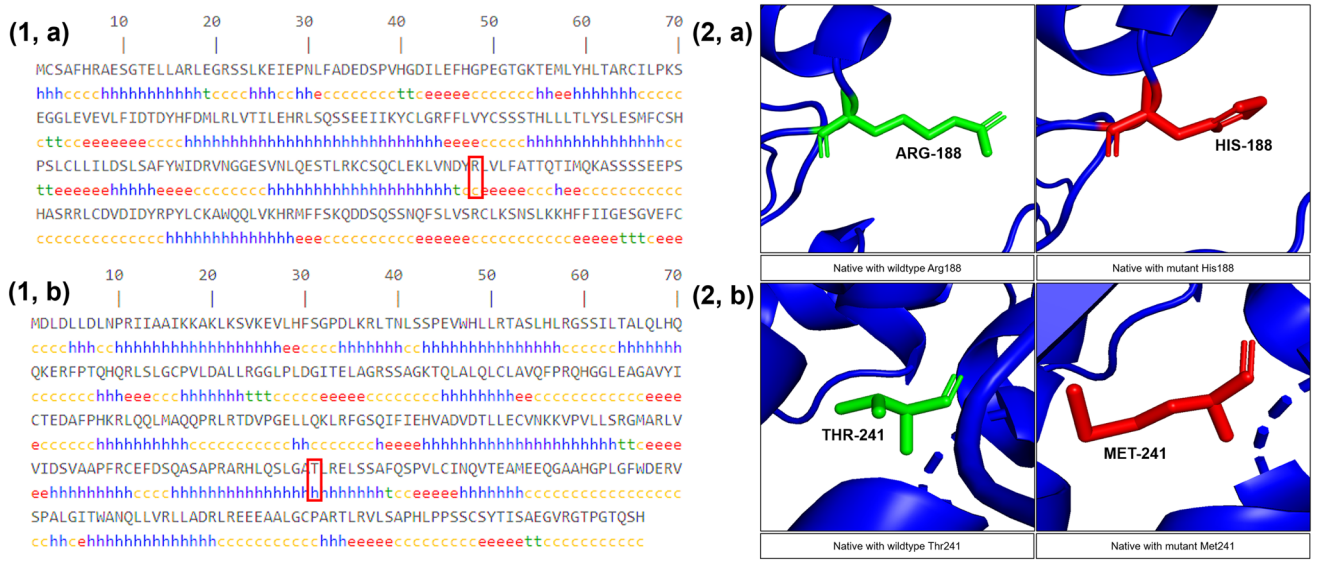

Fig. 4 (1) The secondary structure prediction of individual amino acid of *XRCC2* and *XRCC3* gene by the SOPMA analysis. The boxes indicate the SNPs. (1, a) rs3218536 at 188 position of *XRCC2* (c, Random Coil); (1, b) rs861539 at 241 position of *XRCC3* (h, Alpha Helix). (2) Structure superimposition of *XRCC2* and *XRCC3* proteins. Native and mutant structures were superimposed for the R188H of *XRCC2* and T241M of *XRCC3* gene polymorphisms. (2, a) native with wildtype (Green) and mutant (Red) at R188H position of *XRCC2* gene; (2, b) native with wildtype (Green) and mutant (Red) at T241M position of *XRCC3* gene. (Color figure online)

These selected SNPs were found to have functional significance in previous studies and reported as an important risk factor in the progression of various cancers including OCa by lowering the DNA repair capacity [15, 57, 58]. The current study revealed a significant association between *RAD51* and *XRCC2* gene polymorphisms with OCa risk.

In the literature, various association studies conducted focusing on *RAD51* rs1801320 polymorphism and OCa showed inconclusive results [59–61]. Even though results from studies do not reveal the appropriate role of *RAD51* in cancer development, it was assumed that some of the variants in the 5′ UTR region would help in understanding the role of the *RAD51* gene that could be additive or independent in the progression of OCa [62, 63]. In the present study, the polymorphisms rs1801320 and rs1801321 of the *RAD51* gene revealed a significant association, in which the CC

genotype in rs1801320 and TT genotype in rs1801321 are associated with OCa, which may increase the risk of OCa in the South Indian population. The *RAD51* rs1801320 polymorphism replaces G with C at a 135 position which affects the protein function and translation effect by modifying mRNA splicing [64]. The studies showed that the *RAD51* rs1801320 was found to be associated with the progression of breast cancer and head and neck cancers [50, 65, 66]. Furthermore, rs1801321 showed a significant association with clinical characteristics such as stages and age at menarche. The GT allele was found to be higher in cases diagnosed at the early stages I, and II whereas, the TT allele was found to be higher in stages III and IV. The patients who attain puberty at 13 years of age showed a high frequency of TT allele than the patients attaining puberty at ≤ 13 years of age.

The results have shown that there is a significant association between *XRCC2* rs3218536 polymorphism and OCa risk. The CT and TT alleles showed a strong significant association with OCa. Similarly, in a study conducted in the Polish population, the TT genotype was found to increase the risk of OCa [67]. A meta-analysis showed an association between rs3218536 and OCa but not in other cancers [15]. No significant association was observed between clinical characteristics of OCa patients and *XRCC2* rs3218536 polymorphism.

The *XRCC3* gene polymorphisms did not reveal any association with OCa risk in the South Indian population. A similar result was observed in a meta-analysis of 3,635 cases and 5,473 controls for Thr241Met polymorphism of the *XRCC3* gene [49, 68]. The reported data suggested that the present study on *XRCC3* gene polymorphisms is the first evidence for not revealing the association with OCa risk in the South Indian population. Computational analyses revealed that the missense SNPs of the *XRCC2* (rs3218536) and *XRCC3* (rs861539) genes were having an impact on the protein structure, stability and function.

The observed outcomes for the polymorphisms rs1801320 and rs1801321 of *RAD51* and rs3218536 of *XRCC2* genes in the DNA repair pathway from this study may have an impact on prior diagnosis by using these polymorphisms as markers for OCa screening, which ultimately increases the survival of OCa patients. In conclusion, a direct association was observed between the *RAD51* and *XRCC2* gene polymorphisms and OCa risk in this study. However, some limitations have to be considered before concluding with the observed results. Such studies with a larger sample size are needed with various ethnic groups and should be evaluated with environmental and other risk factors to confirm the association of *RAD51* and *XRCC2* gene polymorphisms with OCa risk. To conclude, results from our study revealed that the polymorphisms in the genes *RAD51* and *XRCC2* are associated and may influence the predisposition of OCa risk in the South Indian population.

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Author contributions All the authors have a substantial role in this manuscript. This study was designed, directed and coordinated by GKG and AMF. GKG was involved in data collection, analysis, interpretation and writing the manuscript. AMF and SFDP helped in drafting the manuscript. JM, MM, NG, RR and UR provided the data for the study and validated results. SS has given input and validated results. All the authors read and approved the final version of the manuscript.

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Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors do not have any conflict of interest to declare.

Ethical approval This work was approved by the Institutional Ethics Committee of Sri Ramachandra Institute of Higher Education and Research (Reference No: IEC-NI/17/JUN/60/80).

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