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Impact of DNA Repair Genes Polymorphisms on Incidence and Prognosis of Breast Cancer in an Egyptian Cohort

Ebtsam R. Zaher[1,*], Mahmoud A. Hemida[2], Mostafa Al-Naggar[3]

[1]Radiation Sciences Department, Medical Research Institute, Alexandria University, Alexandria, Egypt
[2]Experimental and Clinical Surgery Department, Medical Research Institute, Alexandria University, Alexandria, Egypt
[3]Cancer Management and Research Department, Medical Research Institute, Alexandria Universit, Alexandria, Egypt
*Corresponding author: ebtsam.zaher@alexu.edu.eg

Abstract **Background:** Sporadic breast cancer might be caused by low-penetrance genes, including genes constituting the DNA repair pathways. Defective DNA repair is a common imprint of cancer that promotes the accretion of DNA errors and genomic instability. The clustering of damage in DNA may stimulate breast carcinogenesis. **Aims:** The goal of the study is to evaluate the role of single nucleotide polymorphisms in DNA repair genes XRCC1 Arg399Gln, XPD Lys751Gln, RAD51 G135C and XRCC3 Thr241Met as genetic indicators of susceptibility to breast cancer and to evaluate their role in treatment outcome. **Methodology:** The study included 248 females diagnosed with primary breast cancer and 232 normal healthy females. Patients were clinically followed up for 5 years after completing chemotherapy. Genomic DNA was isolated and the four polymorphisms under investigation were assessed by PCR-RFLP technique. **Findings:** XRCC1 399Gln, XPD 751Gln and XRCC3 241Met alleles were significantly associated with breast cancer risk (OR = 2.63, 2.17 and 3.21; respectively), with carriers having lower disease free survival (DSF). When grouping patients based on the number of affected genotypes they carry, DFS decreased as the number of affected genotypes increased (P_{accum}<0.001), patients carrying three (HR=4.74, p<0.001) or two (HR=3.35, p=0.005) affected genotypes had significantly worse DFS compared with those carrying zero (reference) or one (HR=1.37, p=0.093) affected genotype. RAD51 5'UTR G135C polymorphism was not associated with breast cancer risk (p=0.932) or with DFS. **Conclusion:** XRCC1 Arg399Gln, XPD Lys751Gln and XRCC3 Thr241Met polymorphisms may take a significant part in sporadic breast cancer as risk factors and in prognosis, where patients carrying XRCC1 Arg/Arg, XPD Lys/Lys and XRCC3 Thr/Thr genotypes had significantly diminished risk for breast cancer and higher DFS. DFS decreased as the number of affected genotypes increased. But RAD51 5'UTR G135C polymorphism did not associate with either risk or prognosis of breast cancer.

Keywords: *breast neoplasm, DNA repair gene, single nucleotide polymorphism, XRCC1, XRCC3, XPD*

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1. Background

Breast cancer is considered the primary cause of women death worldwide. It represents the highest rates of incidence and mortality of women diseases in Egypt. [1] A huge number of factors were held accountable for the development of breast cancer, which produce amplitude of DNA lesions as oxidized bases, bulky adducts and strand breaks of DNA; these may affect the integrity and stability of DNA but they are effectively corrected by DNA-repair pathways. [2]

Defective DNA repair is a common imprint of cancer that promotes the accretion of DNA errors and genomic instability. The clustering of damage in DNA may stimulate breast carcinogenesis. [3] A complex hierarchy of proteins and enzymes are assigned to detect DNA damage, signal its presence and repair it to protect cells from endogenous and exogenous genotoxic stresses. These may be DNA-damage repair (DDR) genes most importantly, XRCC1 protein which is thought to act as scaffold protein for both single-strand break repair and base-excision repair (BER) activities, and XPD which take part in DNA helix unwinding to allow nucleotide-excision repair (NER) machinery to access the lesion. Also homologous recombinational repair (HRR) genes which act on double-strand breaks and on interstrand crosslinks might be of importance as RAD51 which plays a primary role in repairing double-strand break, and XRCC3 that interacts directly with RAD51, helping in the assemblage of nucleofilament and in selecting and interacting with suitable recombinational substrate. [3,4]

Single nucleotide polymorphism (SNP) is an aberration in DNA sequence which happens when a single nucleotide

differs from the normally expected nucleotide. These SNPs are recognized as regulators for differences in our susceptibility to cancer. SNPs are easy to analyze and required to be analyzed only once, making them interesting biomarkers. SNPs of DDR genes are expected to play a part in the evolution and in the progression of breast cancer. [5]

The goal of the study is to evaluate the role of single nucleotide polymorphisms in DNA repair genes XRCC1 Arg399Gln, XPD Lys751Gln, RAD51 G135C and XRCC3 Thr241Met as genetic indicators of susceptibility to breast cancer and to evaluate their role in treatment outcome.

2. Subjects and Methods

The study included 480 females in two groups; group 1 included 248 females diagnosed with primary breast cancer free of metastasis and Group 2 included 232 normal healthy females matching group 1 in age as a control group. All patients were treated primarily with Modified Radical Mastectomy, [6] followed by adjuvant FAC-based combination chemotherapy (6 cycles each consisted of 5-flourouracil 500 mg/m^2, Adriamycin 50 mg/m^2 and cyclophosphamide 500 mg/m^2, given every 21 days). Patients who were positive for estrogen and progesterone receptors received hormonal therapy (Nolvadex tablets 10mg, 2tablets/day for 5y) after chemotherapy. [7] Patients were clinically followed up for a mean time of 57 months (49-66 months) after completing chemotherapy.

The study was approved by the local ethics committee. After providing an informed written consent, a blood sample was drawn from each subject in EDTA-tubes. The buffy coat was isolated using gradient centrifugation by Ficol-Paque Plus (Biochrom AG, Berlin, Germany). Genomic DNA was isolated from buffy coat using Wizard® SV Genomic DNA Purification System. All the polymorphisms were assessed by PCR-Restriction Fragment Length Polymorphism (RFLP) technique.

PCR was carried out using Go Taq®Green Master Mix (Promega Corporation, Madison, USA). Each PCR reaction mixture consisted of 12.5 µl PCR master mix; 0.1µmol of each amplification primer and 1 µg DNA and the volume was brought to 25 µl by deionized water. Detailed conditions of the PCR and the restriction enzyme digestion and products are listed in (Table 1). PCR products were digested each with its appropriate restriction enzyme (Thermo Scientific™, Thermo Fisher Scientific Inc.). The digested products were separated by electrophoresis in 3% agarose gel, stained by ethedium bromide and observed under UV light for analyzing the genotyping of genetic polymorphisms (Figure 1).

Table 1. Primers, amplification parameters, PCR product, restriction enzymes and corresponding digestion product fragments weight used for RFLP analysis.

Conditions	XRCC1 Arg399Gln	XPD Lys751Gln	XRCC3 Thr241Met	RAD51 G135C
PCR Primers	F: 5'-CAA GTA CAG CCA GGT CCT AG-3' R:5'-CCT TCC CTC ATC TGG AGT AC-3'	F:5′-ACT TCA TAA GAC CTT CTA GC-3′ R:5′-GAT TAT ACG GAC ATC TCC AA-3′	F: 5'-GCC TGG TGG TCA TCG ACT C-3' R: 5'-ACA GGG CTC TGG AAG GCA CTG CTC AGC TCA CGC-3'	F: 5'-TGG GAA CTG CAA CTC ATC TGG-3' R: 5'-GCG CTC CTC TCT CCA GCA G-3'
Annealing	58°C/30 s	55° C / 30 s	60°C /30 s	54°C / 30 s
No. of cycles	35	35	35	35
Product (bp)	268	326	136	175
Restriction enzyme	BcnI 37° C / 10 h	EarI 37° C / 4 h	NlaIII 37° C / O.N.	MvaI 37° C / 6 h
Digestion product (bp)				
W	91 and 177	127 and 199	136	86 and 71
M	268	326	35 and 101	175

Bp: base pairs, W: wild-type, M: mutated.

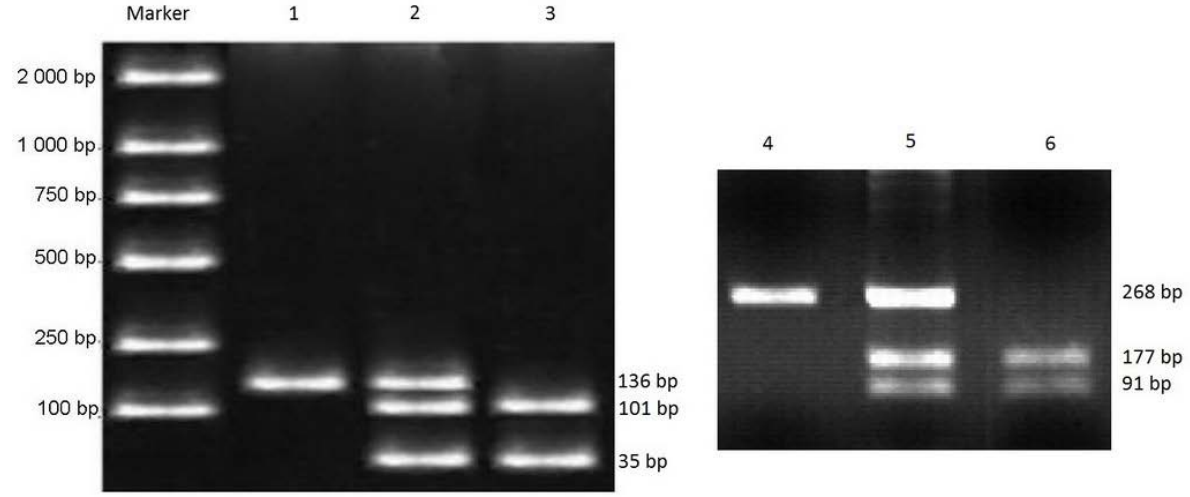

Figure 1. Gel electrophoresis of enzymatically-digested PCR products of XRCC3 and XRCC1 genes, showing lanes numbers: 1, Thr/Thr; 2, Thr/Met; 3, Met/Met; 4, Gln/Gln; 5, Arg/Gln and 6, Arg/Arg genotypes

2.1. Statistical Analysis

All statistical analyses were evaluated by the Statistical Package for Social Sciences software (SPSS version22.0; SPSS Inc.; Chicago, IL, USA). The Hardy–Weinberg equilibrium (HWE) in the frequencies of allele and genotype, and general characteristics between breast cancer patients and cancer-free controls were analyzed by chi-square test. The odd ratios (ORs) and 95% confidence intervals (95%CIs) from unconditional logistic regression were utilized to evaluate the potential associations between genetic variants of DNA-repair genes and the risk of breast cancer. P value less than 0.05 was defined as statistically significant. Disease-free survival (DFS) was calculated from the day of surgery until recurrence or last follow-up. The survival estimates were calculated using the Kaplan–Meier method. The differences in DFS across different genotypes were compared using the log-rank test. Hazard ratios (HR) and 95% CI were estimated using univariate Cox proportional hazards models.

3. Results

The study included 248 breast cancer patients and 232 females as controls. The clinicopathological characterization of the patients' group is presented in Table 2.

The age of the cohort ranged from 37 to 74 years, with a median of 48 years. The mean tumor size was 4.6 cm with an SD of 3.4 cm. Half the cases were of histological grade II with only 8.9% of grade I, but regarding the clinical stage, the majority of cases (48.4%) were of grade III. Most of the cases were positive for estrogen (71.0%) and progesterone (66.5%) receptors. More than half the cohort showed positive lymph node involvement (56.5%) and vascular invasion (57.3%).

The genotype and allele frequencies of DNA repair polymorphisms studied for breast cancer cases and controls are presented in Table 3.

Concerning XRCC1 Arg399Gln and XRCC3 Thr241Met polymorphisms, the allele frequencies between breast cancer patients and their respective controls were significantly different (p=0.034 and 0.006 for XRCC1 and XRCC3; respectively). Also the genotype frequencies in breast cancer patients were significantly different than in controls (p=0.003 and 0.003 for XRCC1 and XRCC3; respectively).

As for XPD Lys751Gln polymorphism, a significant difference in genotype frequencies was found between cancer and control groups (p=0.046). However, the allele frequencies in breast cancer patients were not significantly different from that in controls (p=0.068). RAD51 5'UTR G135C polymorphism did not show any significant difference between breast cancer patients and controls neither in allele frequencies (p=0.887) or in genotype frequencies (p=0.682).

Table 2. Description of the clinicopathological features of breast cancer patients

Characteristic		Breast cancer (248) Number (%)
Age (years)	**median**	48
	Range	37 - 74
Tumor Size	**(Mean± SD) cm**	4.6 ± 3.4
Histological Grade	**I**	22 (8.9)
	II	125 (50.4)
	III	101 (40.7)
Clinical Stage	**I**	22 (8.9)
	II	106 (42.7)
	III	120 (48.4)
Estrogen receptor	**-ve**	72 (29.0)
	+ve	176 (71.0)
Progesterone receptor	**-ve**	83 (33.5)
	+ve	165 (66.5)
Lymph node invasion	**absent**	108 (43.5)
	Present	140 (56.5)
Vascular invasion	**absent**	106 (42.7)
	Present	142 (57.3)

Table 3. Genotype and allele frequencies of polymorphisms inXRCC1, XPD, RAD51 and XRCC3 genes in breast cancer patients and controls

Polymorphism	Genotype /Allele	Breast cancer		Controls		X^2	p
		No.	%	No.	%		
XRCC1 Arg399Gln	Arg / Arg	82	33.1	118	50.9	11.039	0.003
	Arg / Gln	130	52.4	86	38.1		
	Gln / Gln	36	14.5	28	12.0		
	Arg	294	59.3	322	69.4	5.375	0.034
	Gln	202	40.7	142	30.6		
XPD Lys751Gln	Lys / Lys	114	46.0	124	53.4	3.794	0.046
	Lys / Gln	102	41.1	86	37.1		
	Gln / Gln	32	12.9	22	9.5		
	Lys	330	66.5	334	72.0	2.803	0.068
	Gln	166	33.5	130	28.0		
XRCC3 Thr241Met	Thr / Thr	90	36.3	132	56.9	10.613	0.003
	Thr / Met	112	45.2	68	29.3		
	Met / Met	46	18.6	32	13.8		
	Thr	292	58.9	332	71.6	8.691	0.006
	Met	204	41.1	132	28.4		
RAD51 5'UTR G135C	GG	206	83.1	194	83.6	0.916	0.682
	GC	38	15.3	34	14.7		
	CC	4	1.6	4	1.7		
	G	458	90.7	422	90.9	1.131	0.887
	C	24	9.3	42	9.1		

Table 4 represents the association between polymorphism in DNA-repair genes and breast cancer. For XRCC1 Arg399Gln polymorphism, compared to the Arg/Arg genotype, 399Gln allele was significantly associated with breast cancer (p=0.006) with an OR of 2.63 (CI: 1.31-4.25). Likewise, in XPD Lys751Gln, compared to Lys/Lys genotype, 751Gln allele was significantly associated with breast cancer (p=0.012) with an OR of 2.17 (CI: 1.19-3.98). Also regarding XRCC3 Thr241Met, compared to the Thr/Thr genotype, 241Met allele was significantly associated with breast cancer (p=0.001) with an OR of 3.21 (CI: 1.42-4.14). As for RAD51 5'UTR G135C polymorphism, no association as found between 135C allele and breast cancer compared to the GG genotype (p=0.932).

After follow up of breast cancer patients for 5 years, 170 patients (68.5%) remained free while 78 patients (31.5%) relapsed. Patients were analyzed for DSF according to each of the studied polymorphisms (Figure 2). Breast cancer patients carrying XRCC1 399Gln allele had a significantly lower DFS than XRCC1 Arg/Arg genotype carriers (22.4 vs 48.1 months; p<0.001), Also XPD 751Gln allele carriers had a significantly lower DFS than XPD Lys/Lys genotype carriers (26.2 vs 39.9 months; p=0.026), and XRCC3 241 Met allele carriers had a significantly lower DFS than XRCC3 Thr/Thr genotype carriers (46.2 vs 23.1 months; p<0.001). Meanwhile RAD 51 polymorphism did not reflect any significant difference on DFS of breast cancer patients (p=0.886).

Table 4. Association between polymorphism in DNA-repair genes and breast cancer

Polymorphism	Genotypes	OR (95% CI)	X^2	p
XRCC1 Arg399Gln	Arg/Arg vs	1.00	7.438	0.006
	Arg/Gln + Gln/Gln	2.63 (1.31-4.25)		
XPD Lys751Gln	Lys/Lys vs	1.00	5.326	0.012
	Lys/Gln + Gln/Gln	2.17 (1.19-3.98)		
XRCC3 Thr241Met	Thr/Thr vs	1.00	10.517	0.001
	Thr/Met + Met/Met	3.21 (1.42-4.14)		
RAD51 5'UTR G135C	GG vs	1.00	0.173	0.932
	GC + CC	1.02 (0.61-1.71)		

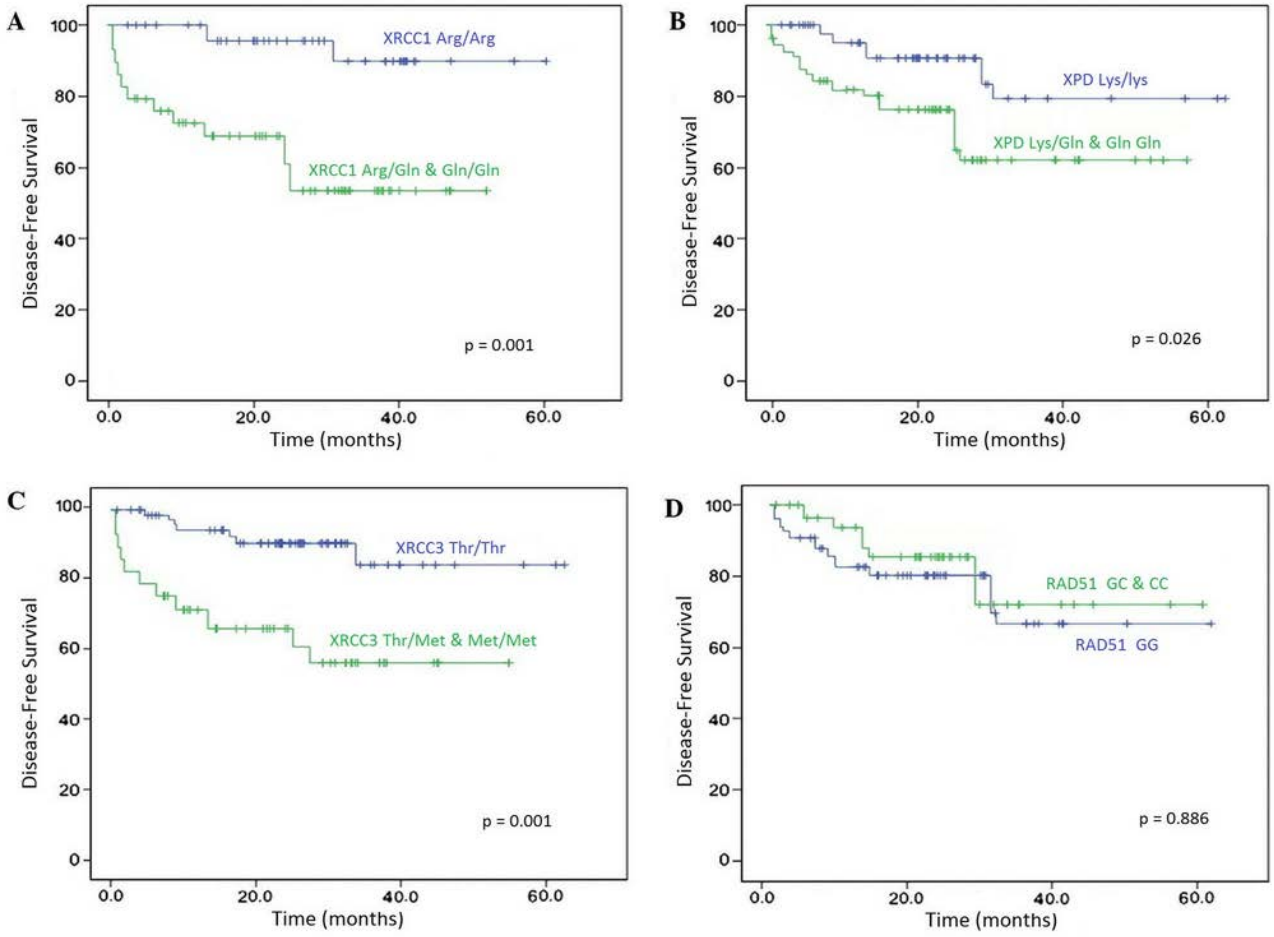

Figure 2. Kaplan-Meier disease-free survival curves of breast cancer patients according to XRCC1 (A), XPD (B), XRCC3 (C) and RAD51 (D) genotypes

Because the XRCC1 399Gln, XPD 751Gln, and XRCC3 241Met alleles were associated with worse survival outcomes, we considered the presence of any one of these three alleles as altered genotype and then evaluated their combined effects by grouping patients based on the number of affected genotypes they carry. DFS was decreased as the number of affected genotypes increased (p_{accum} <0.001). Based on multivariate analysis, patients carrying two (HR=4.74, p<0.001) or three (HR=3.35, p=0.005) affected genotypes had significantly worse DFS compared with those carrying zero (reference) or one (HR=1.37, p=0.093) affected genotype (Table 5 and Figure 3).

Table 5. Combined effects of the three significant polymorphisms on disease-free survival

No. of altered genotypes	No. of patients (%)	5-year survival rate	HR (95% CI)	*p*
0	24 (9.7 %)	91.6 %	1.00	-
1	82 (33.1 %)	87.8 %	1.37 (0.68-3.57)	0.093
2	98 (39.5 %)	61.2 %	3.35 (1.40-5.77)	0.005
3	44 (17.7 %)	36.4 %	4.74 (1.92-9.98)	<0.001
P_{accum}			<0.001	

P_{accum}: significance of the effect of accumulation of affected genotypes.

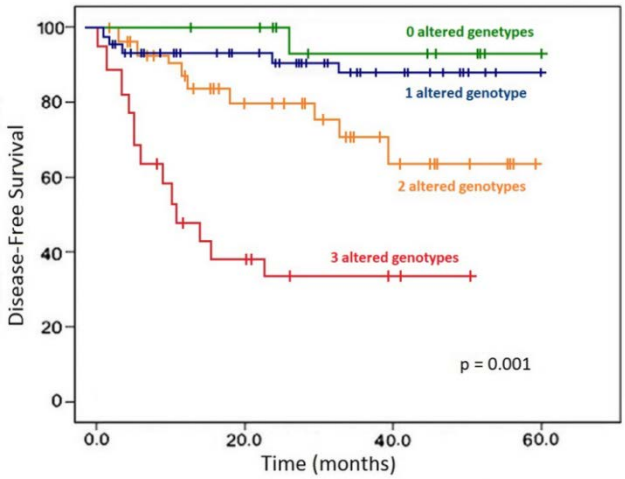

Figure 3. Kaplan-Meier disease-free survival curves of breast cancer patients according to number of altered genotypes

4. Discussion

Breast cancer is a perplexing trait caused by factors that might be environmental or genetic. Multiple environmental factors were identified as risk factors in breast cancer, including age at menarche, first birth and menopause, oral contraceptives, exogenous hormone, fat-rich diet, and family history, but the genetic foundation underlying the disease remained largely unknown. [2] Therefore, research is currently in progress to identify the molecular markers which are related to breast cancer progression and prognosis. Single nucleotide polymorphism (SNP) is the most familiar pattern of genetic alteration in human. Some SNPs affect enzymatic expression or activities, and therefore are connected to the risk, progression or prognosis of cancer. [8]

DNA repair systems are essential to conserve the integrity of the genome, which is crucial for normal cellular growth and differentiation. [4] There is increasing data supporting that genetic polymorphisms of various DNA repair genes result in reduced capacity of DNA repair, inducing accumulation of DNA damage and gene mutations. In this way, the deregulation of DNA repair genes can dramatically increase susceptibility to various human solid tumors. [5]

In humans, more than 125 genes are involved in the response to DNA damage composing five systems for DNA repair: direct reversion of damage, base-excision repair (BER), nucleotide-excision repair (NER), mismatch repair (MMR), homologous recombination repair (HRR) and non-homologous DNA end joining (NHEJ). [4] The impact of polymorphisms in DNA repair genes on cancer risk were extensively investigated, but a few reports evaluated their prognostic significance.

BER is implicated in the repair of base damage issued from exposure to X-rays, oxygen radicals or alkylating agents. [4] In the BER pathway, the X-ray repair cross-complementing group 1 (XRCC1) gene encodes a scaffolding protein that is implicated in the repair of single-strand breaks. Three recognizable domains of XRCC1 are sites for interaction with DNA polymerase b, poly (ADP-ribose) polymerase (PARP), and DNA ligase III. [9]

Within the coding region of XRCC1, the non-synonymous polymorphism, Arg399Gln, has caught much attention in breast cancer risk. This polymorphism is located in the critical COOH-terminal side of PARP-binding BRCA1 carboxyl-terminal-domain (BRCT-domain). [10] The amino acid substitution caused by this variant has been shown to change XRCC1's structure and prevent the combination of BRCT1 and PARP1, which completely disrupt XRCC1 function, resulting in diminished DNA repair capacity. [11]

Our findings suggest that the XRCC1 399Gln allele, modeled as a dominant trait, is a significant risk factor for breast cancer, posing a 2.63 fold breast cancer risk on its carriers. The Arg399Gln gene polymorphism has been associated with breast cancer in many studies with different magnitude of risk in different population or categories of patients. Individuals who had the Gln allele were reported to be more prone to breast cancer under different genetic model (dominant, recessive and additive). [11,12,13] Yet controversial results were reported, as many studies reported no association between breast cancer and XRCC1 gene polymorphisms, [14] or even decrease of breast cancer risk. [15]

NER pathway removes a huge variety of damage to the human genome. [4] The important element of NER, Xeroderma Pigmentosum group D (XPD), or excision repair cross-complementing group 2 (ERCC2), is a conserved ATP-dependent helicase that serves two functions: nucleotide excision repair and basal transcription as part of transcription factor complex, TFIIH. XPD participates in the opening of the DNA helix to allow the excision of the DNA fragment containing the damaged base, thus playing a key role in the elimination of certain DNA cross-links, ultraviolet-caused photolesions, and bulky chemical adducts. [16] XPD Lys751Gln polymorphism is a frequent SNP of the XPD locus that lead to amino acid substitution. The A to C substitution at codon 751 entails a drastic change in charge configuration of the resulting amino acid

and diminished DNA repair efficiency, which was reported to underlie an upraised risk for several kinds of solid tumors. [17]

The current study indicates a protective role of XPD Lys/Lys genotype leaving XPD Gln allele carries with 2.17-fold increased risk of breast cancer. Controversial and even contradictory results were reported on the association of XPD Lys751Gln polymorphism with breast cancer risk, [18] however, several studies and meta-analysis were in agreement with our data and reported an increased risk associated with XPD 751Gln allele, although with varying magnitudes of risk. The risk probably varied in association with different ethnic groups as the reported risk was higher for Caucasian females but with lower magnitude for Asian and African populations. [16,17,19]

Double-strand break is the commonest form of DNA damage initiated by radiation and its repair can be done by two pathways; HRR and NHEJ. The XRCC3 (X-ray repair cross-complementing group 3) encodes an element of the RecA/Rad51-related protein family which contributes to HRR in order to repair DNA damage and preserve chromosome stability. [20] Concern has been raised about the association of Thr241Met with breast cancer risk, but the results remain controversial rather than conclusive. At the meta-analytical level, Thr241Met polymorphism was related to increased risk of breast cancer when all investigations were grouped together. [20,21,22] That was in accordance with the results from the current work, where XRCC3 241Met was associated with increased risk for breast cancer, posing a 3.21 fold upraised risk on its carriers.

RAD51 is the homolog of bacterial RecA protein that plays an essential role in preserving stability of the genome. It serves as a core element in the homolog-dependent recombinational repair of the double strand breaks through assembled nucleoprotein filaments on single stranded DNA. It in turn mediates the strand invasion and exchange between the homologous DNA and damaged site. [23] RAD51 reacts with BRCA1 and BRCA2 for HRR. A functional polymorphism in position 135 of 5'-untranslated region in RAD51 gene, substituting a guanine to cytosine, has been accused of modulating breast cancer risk by modifying gene transcription. The G135C polymorphism affects mRNA stability and in turn translation efficiency, thus it generates altered products, which further influence the functionality of a multi-protein DNA repair complex composed of BRCA1, BRCA2 and RAD51. [24]

Many epidemiological studies were done to examine the association of RAD51 G135C polymorphism with the risk of breast cancer in different populations. But, inconsistent results, even within the same population, have hindered the consensus building regarding the impact of this polymorphism on breast cancer risk. Interestingly, even meta-analyses presented different outcomes, as the conclusion of the meta-analyses on RAD51 G135C varies from one extreme of association, [23,24,25] to another extreme of no association, [24] or even suggesting a protective impact of the SNP. [26]

The current study failed to prove association between RAD51 polymorphism with risk of breast cancer. This could be in accordance with meta-analyses reporting no risk association with this SNP, or those reporting that the risk is strongly affected by ethnicity of the studied population, since group-wise analysis indicated strong effect of this polymorphism in Caucasians, though not in East Asians or in populations of mixed ethnicities. [25,27]

However, a huge inconsistency and even discrepancy across the studies exist whenever a SNP is being evaluated for cancer risk. This might be due to a large number of factors that includes study design, sample size, criteria for recruitment of cases and controls, cancer-specific factors, statistical tests applied, and above all ethnicities of study populations.

After follow up of breast cancer patients for 5 years, 170 patients (68.5%) remained free while 78 patients (31.5%) relapsed. Disease-free survival (DSF) was evaluated in patients in accordance with each of the studied polymorphisms. Breast cancer patients having XRCC1 399Gln, XPD 751Gln and XRCC3 241 Met alleles had a significantly lower DFS while RAD51 G135C polymorphism did not affect DFS of breast cancer patients. Our results were in accordance with other studies concerning the impact of these repair genes on survival of patients with breast cancer. [28,29]

The most significant finding of this study was that combined analysis of multiple SNP in the same or relevant pathways had a much higher potential value in terms of prognostic importance. Three of the studied SNPs were associated with survival outcome in individual SNP analysis, however, when these three SNPs were combined, DFS decreased as the number of affected genotypes increased (p_{trend} <0.0001). Matullo et al. [30] reported a dose-dependent relationship between the number of unfavorable alleles in DNA repair gene SNPs and the level of DNA adducts in peripheral blood cells, indicating a stepwise decline in DNA repair capacity as the number of unfavorable alleles increase, and providing biological plausibility and validity to our approach.

The present data suggests that polymorphisms of DNA repair gene could affect malignant phenotypes. There is an increasing recognition that genetic polymorphisms strongly impact not only the development of cancer, but also its progression and prognosis [5]. DNA repair gene polymorphisms were demonstrated to correlate with DNA repair capacity which leads to clonal expansion of abnormal cells through the acquisition of proliferative advantages. Consequently, it is a possibility that functional polymorphisms in DNA repair genes might impact the natural history of cancer, such as tumor histopathology, disease progression, or propensity for metastasis, thereby influencing survival outcome.

In conclusion, the results of the study suggest that XRCC1 Arg399Gln, XPD Lys751Gln and XRCC3 Thr241Met polymorphisms are expected to take a significant part in sporadic breast cancer progression as risk factors and in patients' prognosis, where patients carrying XRCC1 Arg/Arg, XPD Lys/Lys and XRCC3 Thr/Thr genotypes had a significantly diminished risk for breast cancer and higher DFS. When these three SNPs were joined, DFS decreased as the number of affected genotypes increased (p_{accum} <0.0001). But RAD51 5'UTR G135C polymorphism did not associate with either risk or prognosis of breast cancer.

Statement of Competing Interests

The authors have no competing interests.

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