Association of *XRCC1* Arg399Gln and *RAD51* 135 G>C Polymorphisms and Epidemiologic Risk Factors with Colorectal Cancer among Selected Filipinos

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ABSTRACT

Objectives. Several studies have demonstrated that genetic variants of certain DNA repair genes such as the *RAD51* and *XRCC1* increase cancer risk substantially. The results were also observed to be race- and tumor site-specific. Hence, this study aimed to determine the possible association of *XRCC1* Arg399Gln and *RAD51* 135G>C polymorphisms combined with risk factors of colorectal cancer (CRC) among selected Filipinos.

Methods. Genomic DNA isolated from peripheral blood samples of histologically confirmed CRC patients (n=70) and their age- and sex-matched clinically healthy controls (n=70) were analyzed for polymorphisms of *XRCC1* and *RAD51* genes by polymerase chain reaction.

Results. The genotypic distribution pattern of *RAD51* 135G>C (p>0.05) was not significantly different between the CRC cases and controls. Significantly higher incidence (*p*=0.016) of the *XRCC1* GG genotype was noted among the cases (n=34, 49%) compared with controls (n= 20, 29%). Individuals carrying the *XRCC1* AG genotype have a lower risk of developing CRC (OR=0.42, 95% CI=0.21-0.85) than the *XRCC1* GG genotype. *XRCC1* AG genotype combined with alcohol drinking, smoking, or family history of cancer also showed a lower risk of developing CRC. There was no significant association between the genetic variants of *RAD51* 135G>C and CRC risk. Carriers of both *XRCC1* GG and *RAD51* CC genotypes showed a 5x higher risk (OR=5.02; 95%; CI=1.0429-24.1283) compared to those carrying other genotype combinations (*p*=0.028).

Conclusions. *XRCC1* Arg399Gln but not *RAD51* 135G>C may be associated with CRC development among Filipinos. Individuals who drink alcohol, smoke tobacco and have a family history of cancer have a lower risk of developing CRC when they are also carrying the *XRCC1* AG genotype. The findings may have significant implications in designing personalized methods for screening, diagnosing, and treating CRC.

Keywords: XRCC1, RAD51, genetic polymorphisms, colorectal cancer, PCR

INTRODUCTION

More than 1.8 million new cases of colorectal cancer (CRC) resulting in 881,000 deaths have been recorded worldwide, making it the third most diagnosed cancer and the second leading cause of cancer-related deaths in 2018.[1,2] By 2030, CRC is projected to rise by 60% with over 2.2 million new cases and 1.1 million deaths.[3] The Global

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Cancer Observatory (GLOBOCAN) ranks CRC as the third most common cause of cancer in the Philippines, with >15,000 new cases and >9,000 deaths in 2018.[4]

CRC generally starts with transforming a normal colorectal epithelium to a benign adenomatous polyp that may lead to colorectal adenocarcinoma. The latter develops through the stepwise accumulation of multiple genetic and epigenetic anomalies, which then progresses to invasive and metastatic tumors.[5,6] Genomic instability and the resulting gene alterations are key molecular pathogenic steps that occur early in carcinogenesis. These allow the acquisition of a sufficient number of alterations in tumor suppressor genes and/or activation of oncogenes that transform cells and promote tumor progression. The DNA repair pathways work to inhibit anomalies during DNA replication that may result in malformations or carcinogenesis. However, certain genetic variants of DNA repair genes such as the *RAD51* and *XRCC1* may substantially increase the risk of cancer in carriers because of biochemical alterations resulting from these polymorphisms.[7,8]

RAD51 plays a central role in the homologous recombination of DNA during double-strand break repair. Its protein product catalyzes strand transfer and mediates homologous pairing for the resynthesis of the damaged region.[9–11] *XRCC1*, on the other hand, is one of the most important genes involved in base excision repair (BER) and single-strand break repair processes. Its scaffolding protein product is strongly associated with BER pathway coordination by interacting with most BER short patch pathway components.[8,12,13] Several studies have shown that single nucleotide polymorphisms (SNPs) of *XRCC1* are linked with measurably reduced DNA repair capacity and increased risk of several types of cancers, including CRC.[13]

Studies on the possible association *of RAD51* G135C and *XRCC1* Arg399Gln with the risk of various cancers have been proven to be race- and tumor site-specific.[7,14–33] *RAD51* G135C is associated with CRC development among Turkish[7], Bangladeshi[17], and ethnic Kashmiri[20] but not among the Iranians.[34] It is also associated with breast cancer development among Saudi females[28], Chinese[35], and Filipino[36] populations but not among the Finnish population.[37] *XRCC1* Arg399Gln is associated with CRC development among Kashmiri[38], S. Koreans[27], Japanese[39], southwest Iranians[40] but not among Malaysians[41], and Mexican cohorts.[15] Both *XRCC1* Arg399Gln and *RAD51* G135C have been associated with ovarian cancer among Serbian women.[30]

Therefore, this case-control study aimed to determine the possible association of *XRCC1* Arg399Gln and *RAD51* 135G>C polymorphisms combined with other epidemiologic risk factors like smoking, alcohol drinking, and history of cancer in both immediate and extended family with CRC development among selected Filipino population. The findings may have significant implications in designing personalized methods for screening, diagnosing, and treating CRC.

MATERIALS AND METHODS

Ethical clearance

Ethical clearances were secured from the Institutional Review Boards of the University of Santo Tomas Hospital (USTH) in Manila (Protocol Reference Number: IRB-2016-12-201-IS) and Mariano Marcos Memorial Hospital and Medical Center (MMMH-MC) in Ilocos Norte (RERC Protocol Number: MMMH-RERC-17-002), Philippines, before the implementation of the study. All the study participants gave their written informed consent.

Study Participants

Filipino patients, who are not products of interracial marriages of both parents and grandparents, with histologically confirmed colorectal cancer (CRC), aged 18 years old and above, were recruited at USTH from January 2016 to December 2017 and at MMMH-MC from January 2016 to April 2019. The CRC cases were newly diagnosed, receiving treatment, or in remission. They were age- (±2 years) and sex-matched with physician-assessed clinically healthy controls who were not suspected of having any type of malignancy and living in the same geographical location as the cases. Clinicopathologic features were retrieved from medical and histopathological records, and CRC risk factors were obtained through personal interviews.

Genotyping

Genomic DNA was isolated from the study subjects' peripheral blood samples using ReliaPrep™ Blood gDNA Miniprep System (Promega, USA) following the manufacturer's protocol. Genotyping for *XRCC1* was done by polymerase chain reaction with confronting two-pair primers (PCR-CTPP).[14,35] A total PCR reaction volume of 20 µl containing 10µl PCR master mix (Taq DNA polymerase, reaction buffer at pH 8.5, 400µM dNTPs, and 3mM MgCl2), 0.4µl of each primer, 6.4 µl nuclease-free water, and 2 µl of the template DNA was prepared for *XRCC1* SNP analysis. The primers 5'-TCCCTGCGCCGCTGCAGTTTCT-3' (F1), 5'-TGGCGTGTGAGGCCTTACCTCC-3' (R1), 5'-TCGGCGGCTGCCCTCCCA-3' (F2), and 5'-AGCCCTCTGTGACCTCCCAGGC-3' (R2) were used to amplify *XRCC1* under the following conditions: initial cycle at 95˚C for 10 min; 30 cycles of 95˚C for 1 min, 66˚C for 1 min, and 72˚C for 1min; and a final extension at 72˚C for 5 min.[14,35] All PCR products were separated through electrophoresis using 1% agarose gel with SYBR™ Safe DNA gel stain (Thermo Fisher Scientific, Waltham, USA). The expected fragment sizes were 630, 447bp (G/G), 630, 447, 222bp (A/G), and 630, 222bp (A/A).[14,35]

RAD51 genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)[25,42,43] as described with modification on PCR conditions. A total PCR volume of 20 µl containing 10 µl PCR master mix, 0.5 µl of each primer, 7µl nuclease-free water,

and 2 μl of the template DNA. *RAD51* was amplified using the primers 5'TGGGAACTGCAACTCATCTGG-3' (F) and 5'-GCGCTCCTCTCTCCAGCAG-3' (R). The PCR amplification conditions were as follow: initial cycle at 95˚C for 3 min; 40 cycles of 95˚C for 30 sec, 58.7˚C for 30 sec and 72˚C for 30 sec; and a final extension at 72˚C for 5 min. PCR products were then digested using *BstN1* (New England BioLabs, Inc, USA) for 60 min at 60˚C. Digested products were separated in 12% polyacrylamide gel electrophoresis for 90 mins at 100V. Expected digested products were 157 bp (C/C); 157, 86, 71bp (G/C); and 86, 71bp (G/G).

Statistical Analysis

Prevalence of *XRCC1* Arg399Gln and *RAD51* 135 G>C genotypes along with allele and genotype frequencies were computed using the Hardy-Weinberg equilibrium. Pearson's Chi-square test was used to evaluate the genotype frequencies of both *XRCC1* Arg399Gln and *RAD51* 135G>C between the CRC cases and controls as well as the TNM (TNM classification system of malignant tumors) staging and tumor grade differences between all the genotypes. When the expected frequencies were less than five, Fisher's exact test was used. For the ordinal variable, the Wilcoxon rank-sum test was utilized. The association between genotypes and CRC risk factors was estimated as crude odds ratio (OR) with 95% confidence intervals using logistic regression. Statistical analyses were performed using STATA 14 (StataCorp LP, College Station, Texas, USA).

RESULTS

A total of 140 study participants (70 histologically confirmed CRC cases matched with clinically healthy controls) were included. There were no significant differences between cases and controls regarding their epidemiologic risk factors (Table 1).

The genotypic distribution pattern of *XRCC1* Arg399Gln and *RAD51* 135G>C between cases and controls is shown in Table 2. Higher incidence of the *XRCC1 GG* genotype was observed among the cases (*n*=34, 49%) compared with controls (*n*=20, 29%). In contrast, a lower incidence of the AG genotype was observed in cases (*n*=36, 51%) compared to controls (*n*=50, 71%). Genotypic distribution of *RAD51* 135G>C was comparable between cases and controls. Individuals carrying the *XRCC1 AG* genotype showed a significant protective effect against developing CRC (OR=0.42, 95% CI=0.21-0.85). There was no significant association between the genetic variants of *RAD51* 135G>C and CRC risk.

Combining the *XRCC1 AG* genotype with risk factors such as alcohol drinking, smoking, or family history of cancer showed significant protective effect (*p*=0.012-0.016) against developing CRC (Table 3). However, these risk factors did not increase the risk of developing CRC when carrying the *RAD51 GC* genotype. The combined effect of the two SNPs on CRC risk was also determined (Table 4). Individuals carrying both *XRCC1 GG* and *RAD51 CC* genotypes showed a 5x higher risk (OR=5.02; 95%; CI=1.0429-24.1283)

Table 1. Clinical profile of the CRC cases and control participants

Characteristic	Cases		Controls		*p*-value*
	(n = 70)	(%)	(n = 70)	(%)	
Median age at diagnosis (year)	56.2				
Sex					
Male	44	63%	44	63%	>0.999
Female	26	37%	26	37%	
Alcohol drinking					
Former/current	37	53%	36	53%	0.992
Never	33	47%	32	47%	
No answer			2		
Cigarette smoking					
Former/current	31	44%	25	37%	0.368
Never	39	56%	43	63%	
No answer			2		
Family history of cancer (immediate family member)					
Yes	23	33%	13	19%	0.066
No	47	67%	55	81%	
No answer			2		
Family history of cancer (extended family member)					
Yes	13	19%	9	13%	0.392
No	57	81%	59	87%	
No answer			2		

**Chi-square test*

Table 2. Comparison of *XRCC1* Arg399Gln and *RAD51* 135G>C allele and genotype frequencies (n=140)

	Genotype	Cases (n = 70)		Controls (n = 70)		OR (95% CI)	*p*-value*
		Frequency	%	Frequency	%		
XRCC1 Arg399Gln	GG	34	49	20	29	1.00 (ref)	0.016
	AG	36	51	50	71	0.42 (0.21-0.85)	
	AA	0	0	0	0		
	Arg allele	0.74		0.64			
	Gln allele	0.26		0.36			
RAD51 135G>C	CC	17	24	16	23	1.00 (ref)	0.842
	GC	53	76	54	77	0.92 (0.42-2.02)	
	GG	0	0	0	0		
	C allele	0.62		0.61			
	G allele	0.38		0.39			

**Crude odds ratio (OR) with 95% confidence interval was computed using logistic regression*

Table 3. Combined effects of *XRCC1* AG (n=53) and *RAD51* GC (n=106) genotypes with other risk factors

Gene	n	Cases		Controls		OR (95% CI)	*p*-value*
		Frequency	%	Frequency	%		
XRCC1 AG with alcohol use	45	20	44	25	56	0.41 (0.20-0.83)	0.014
XRCC1 AG with smoking	33	16	48	17	52	0.41 (0.20-0.84)	0.015
XRCC1 AG with family history of cancer	29	14	48	15	52	0.40 (0.19-0.81)	0.012
XRCC1 AG with alcohol use and smoking	30	15	50	15	50	0.42 (0.21-0.85)	0.016
XRCC1 AG with alcohol use, smoking, and family history of cancer	12	8	67	4	33	0.40 (0.20-0.83)	0.014
RAD51 GC with alcohol use	58	29	50	29	50	0.88 (0.40-1.95)	0.757
RAD51 GC with smoking	47	26	55	21	45	0.83 (0.37-1.86)	0.660
RAD51 GC with family history of cancer	31	19	39	12	61	0.98 (0.44-2.21)	0.964
RAD51 GC with alcohol use and smoking	43	24	56	19	44	0.82 (0.37-1.84)	0.639
RAD51 GC with alcohol use, smoking, and family history of cancer	14	11	79	3	21	0.84 (0.40-2.09)	0.835

**Crude odds ratio (OR) with 95% confidence interval was computed using logistic regression*

compared to those having other genotype combinations analyzed in this study (p=0.028). Lastly, results showed no association of the *XRCC1* and *RAD51* genotypes with the tumor grade or the TNM stage (Table 5).

DISCUSSION

This case-control study analyzed the *XRCC1* Arg399Gln and *RAD51* 135G>C polymorphisms to determine their association with CRC development among Filipinos. Results showed that *XRCC1* Arg399Gln but not *RAD51* 135G>C might be associated with CRC development among Filipinos. Furthermore, the combination of *XRCC1 GG* and *RAD51 CC* genotypes showed a 5x higher risk of developing CRC. This showed that there was a synergistic effect when the two genotypes were combined. Their combination may impact the decreased DNA repair activity, thus contributing significantly to the increased risk of developing CRC.

The DNA is subject to continuous attack from both exogenous and endogenous toxins. Still, mammalian cells have developed several mechanisms specific to each type of damage to repair the damaged DNA.[28] Polymorphic variants of DNA repair genes may influence functional deficiencies in DNA repair processes, leading to increased vulnerability to carcinogenesis.[13,29,30,38] Defects in the DNA repair system would increase the genome's instability and susceptibility to malignant transformation.

The *XRCC1* gene is located on chromosome 19q13.2-13.3. Its encoded protein acts as a scaffolding protein involved in base-excision repair (BER) and single-strand breaks (SSB). It interacts with PARP1, OGG1, and APE1 to facilitate the BER processes.[29] Among its more than 300 poly-

Table 4. Association of combined *XRCC1* and *RAD51* genotypes with CRC development

Combined genotypes	Case	Control	OR (95% CI)	*p*-value*
XRCC1 AG + *RAD51* GC	28	36	0.63 (0.3223-1.2299)	0.175
XRCC1 AG + *RAD51* CC	15	8	0.47 (0.1864-1.2012)	0.110
XRCC1 GG + *RAD51* GC	17	25	1.73 (0.8321-3.6053)	0.140
XRCC1 GG + *RAD51* CC	2	9	5.02 (1.0429-24.1283)	0.028

**Chi-square test except when the expected frequencies were less than five, wherein Fisher's exact test was used*

Table 5. Association of *XRCC1* Arg399Gln and *RAD51* 135G>C genotypes with tumor grade (*XRCC1* n=42, *RAD51* n=42) and TNM staging (*XRCC1* n=59, *RAD51* n=59)

Genotypes		Poorly- and moderately-differentiated tumors		Well-differentiated tumors		*p*-value*	Stage I/II		Stage III/IV		*p*-value*
		n	%	n	%		n	%	n	%	
XRCC1	GG	9	38	8	44	0.650	8	38	18	47	0.492
	AG	15	63	10	56		13	62	20	53	
RAD51	CC	5	21	2	11	0.679	4	19	11	29	0.403
	GC	19	79	16	89		17	81	27	71	

**Chi-square test except when the expected frequencies were less than five, wherein Fisher's exact test was used*

morphisms reported, only three (3) are most widely studied – Arg194Trp, Arg280His, and Arg399Gln.[18,41] These amino acid substitutions influence the protein-protein interactions between *XRCC1* and other BER proteins resulting in altered efficiency, function, and damaged DNA repair capacity leading to genetic instability and carcinogenesis.[13,27,28,31,40,44] Findings regarding the associations of *XRCC1* Arg399Gln with CRC have been inconsistent. It has been associated with CRC among the Kashmiri population[38], South Koreans[27], Han Chinese from Jiangsu Province, East China[45], Japanese[39], and southwest Iranians.[40] However, lack of association has been noted in a Malaysian cohort[41], Han Chinese from the southwest region of China[33], and Mexican patients.[15] The difference in results from the Han Chinese population from two different parts of China has been reported due to differences in the study participants' geographical location and genetic makeup. The Han Chinese subjects of Huang *et al.* (2015)[45] were from Jiangsu Province, which is located in the eastern portion of China, while those of Zhang *et al.* (2014)[33] were from the southwest part of China. Another could be the method of genotyping analysis used by the two research groups. Huang *et al.* performed PCR-RFLP[45] while Zhang *et al.* used PCR-CTPP.[33]

RAD51 plays a central role in repairing double-strand breaks (DSBs) performed by homologous repair (HR). It is the only protein factor that facilitates the pairing and exchange of DNA strands. The *RAD51* gene is located at chromosome 15q14-15 and is highly polymorphic. G to C and G to T substitutions at positions 135 and 172 have been depicted as SNPs at the 5' UTR of the *RAD51* gene, respectively. Both polymorphisms are found in the regulatory element of the *RAD51* promoter and have been suggested to be linked with mRNA stability and expression.[46] The *RAD51* 135G>C polymorphism is the most commonly studied in many cancers, and this polymorphism is linked with increased cancer susceptibility because of its less efficient or defective repair capabilities.[10] *RAD51* is also associated with *BRCA1* and *BRCA2* tumor suppressor gene products, suggesting that a defect in recombination may influence carcinogenesis.[10,32] In the systematic review and meta-analysis of Oh *et al.* (2018)[47], it was pointed out that there is a greater risk of CRC in BRCA1 mutation carriers but not in BRCA2 mutation carriers.

Studies on the association of *RAD51* 135G>C with CRC have varied results. This study showed that there is no association between *RAD51* 135G>C and CRC risk. This is similar to a Polish population study that showed no significant association of *RAD51* 135G>C with CRC development.[43] In contrast, the group of Krupa[15] and Romanowicz[25,32] observed association of this polymorphism with CRC. This discrepancy could be attributed to the difference in sample size[43] or the specimen analyzed. The group of Romanowicz[32] utilized colorectal tissue while Mucha and colleagues[43] used blood. *RAD51* 135G>C has also been associated with CRC among the Bangladeshi[17], Turkish[7,19], and ethnic Kashmiri[20] populations.

Besides being associated with CRC, *RAD51* 135G>C has also been linked with breast cancer development among Saudi females[28] and prostate cancer among Jordanians.[21] In the study of Ragudo *et al.* (2020)[36], the more common *RAD51* G/C genotype was not associated with breast cancer development among Filipinos, while the less common recessive C/C genotype was observed to potentially increase the risk. *XRCC1* Arg399Gln has been associated with breast cancer among Serbian women[22] and lung cancer among Chinese.[23] Both *RAD51* 135G>C and *XRCC1* Arg399Gln have been associated with ovarian cancers among Serbian women.[30] A meta-analysis on *RAD51* 135G>C SNPs showed significant association with increased risk of squamous cell carcinoma of the head and neck (SCCHN)

but not in ovarian cancer, acute leukemia, and CRC.[24] This is consistent with the present study results regarding the lack of association of *RAD51* 135G>C with CRC.

Alcohol consumption, tobacco smoking, and family history of cancer have been reported to increase the risk of CRC development.[16,39] Alcohol consumption and smoking have been established as rich sources of reactive oxygen species and chemical carcinogens known to damage DNA.[27,45] Although tobacco smoking has long been known to cause lung cancer; studies show that smoking is also detrimental to the colon and rectum because of its carcinogen content. Regular heavy alcohol consumption is also associated with developing a disproportionate increase of tumors in the distal colon. Combined smoking and alcohol consumption can induce chronic inflammation, further increasing the risk of CRC development.[48,49] Alcohol can act as a co-carcinogen by enabling absorption of carcinogens or as carcinogen itself by converting acetaldehyde in the lumen of the colon and induce DNA damage.[45] Results of the study showed that the *XRCC1 AG* allele has a protective effect even with these risk factors.

Interestingly, the study of Skjelbred *et al.* (2006)[50] also showed a reduced risk of high-risk adenomas (a benign form of colorectal tumor) in persons with the *XRCC1* Gln allele. The protective effect of the polymorphism may be due to the reduced DNA repair activity of cells, thus encouraging apoptosis instead of repair. Apoptosis is triggered when DNA breaks are not repaired.[51]

This study's findings showed no correlation of the tumor grade or TNM stage with *RAD51* 135G>C or *XRCC1* Arg399Gln polymorphisms. It may be interesting to include the possible association of polymorphisms in these genes with survival and treatment response in future studies. *XRCC1* Arg399Gln has been observed to have no association with survival among Thai CRC patients.[16]

In conclusion, *XRCC1* Arg399Gln but not *RAD51* 135G>C may be associated with CRC development among Filipinos. Individuals who drink alcohol, smoke tobacco, and have family history of cancer have a lower risk of developing CRC when they are also carrying the *XRCC1 AG* genotype. Despite the small number of study participants, the strict age, sex, and geographical origin-match design add to the reliability of this study's findings. However, it is recommended that future studies should include a larger number of participants and include subjects from other parts of the country to represent the Filipino population accurately. Also, *BRCA1* mutation in study participants may be determined since the association of *RAD51* with the *BRCA* genes suggests that its homologous activity is affected. Other genetic polymorphisms associated with CRC development among Filipinos should also be screened and utilize other analytes like formalin-fixed paraffin-embedded (FFPE). Fresh tissues can have significant implications in designing personalized methods for screening, diagnosing, and treating CRC.

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Statement of Authorship

All authors participated in the data collection and analysis and approved the final version submitted.

Author Disclosure

All authors declared no conflicts of interest.

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