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ORIGINAL ARTICLE

Impact of a Missense Variation (p.S150R:AGC>AGG) in the *XRCC2* Gene on Susceptibility to Colorectal Cancer

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SUMMARY

Background: **The X-ray repair complementing defective repair in Chinese hamster cells 2 (XRCC2) is an important protein in response to DNA double-strand breaks (DSBs) in human cells. XRCC2, as a functional protein in the homologous recombination repair (HRR) process, has been identified to have several polymorphisms which might be associated with the risk of cancer. Therefore, we aimed to investigate a novel missense variation (AGC>AGG, p.Ser150Arg) in the *XRCC2* gene for colorectal cancer susceptibility.**
Methods: **We studied 291 colorectal cancer (CRC) patients and 140 healthy individuals. ARMS PCR method was used to detect the AGC>AGG (p.Ser150Arg) variation in the *XRCC2* gene.**
Results: **The results showed that there was a significant differential among CRC and controls in the genotypic and allelic frequencies ($p < 0.001$) of *XRCC2*; AGC>AGG, p.Ser150Arg. Our results demonstrated that the G allele of *XRCC2*; AGC>AGG, p.Ser150Arg was associated with increased CRC risk (odds ratio = 59.04, 95% confidence interval = 18.6 - 186). This variation also influenced CRC cancer susceptibility in smokers ($p < 0.001$).**
Conclusions: **The G allele of *XRCC2*; AGC>AGG, p.Ser150Arg, may be a potential marker for CRC in Iranians and investigations in other populations are warranted for further universal application in CRC detection and prediction.**
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INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers leading to over 608,000 deaths (8% of overall cancer) annually worldwide. Although epidemiology data show that almost 60% of cases occur in Western population, it is still a major health problem in developing countries. According to previous studies, the number of new cases of CRC in Iran is 3,641 per year, which is approximately 6.3% of all cancer deaths in Iran [1-6]. The risk of developing CRC is influenced by both

genetic and environmental factors. Chemicals, radiations and some endogenous elements can obviously cause damage to DNA. As a result, single strand breaks (SSBs) occur and, subsequently, unrepaired single strands lead to double strand breaks (DSBs) during the S phase of the cell cycle [7]. It has been shown that accumulation of unrepaired DSBs can lead to cell death and initiate malignancies, which highlights the disorder of DNA repair as the key role in tumorigenesis [8]. There are several DSB repairing mechanisms, among which homologous recombination repair (HRR) is the leading functional pathway in the S phase of the somatic mammalian cell cycle [8]. Faulty HRR has been reported to be closely associated with human cancers [9].
It has been recently demonstrated that RAD51 paralogs such as XRCC2 could serve as central proteins during the HRR process [10]. XRCC2 protein in combination with other proteins, such as RAD51L3 [11], makes a complex which plays a critical role in the apoptotic response to DSBs and chromosome segregation [12]. Many studies have found that single nucleotide polymorphisms (SNPs) in the *XRCC2* gene have an impact on the DNA repair capacity and the lack of appropriate function of this repairing gene can cause cancer formation including colorectal cancer [13]. Johnson et al. detected an over 100-fold reduction of HRR in the XRCC2 deficient hamster cells compared to the parental cells [14], which proved the essential function of XRCC2 protein for HRR process.
In this study, for the first time, we investigated a novel missense variation (AGC>AGG, p.Ser150Arg) in the *XRCC2* gene to susceptibility on colorectal cancer.

MATERIALS AND METHODS

Subject Population

This case-control study of colorectal cancer was carried out from 2014 to 2017. Blood samples were obtained from a total of 431 Iranian individuals including 291 colorectal cancer patients (153 males and 138 females) and 140 healthy persons (57 males and 83 females) who did not have any identified cancer profiles. These Iranian patients were examined by colonoscopy and histology of biopsies, and histologically they had confirmed invasive adenocarcinoma of the colon. Also, they were not associated with an inherited cancer syndrome. All demographic data were included by the specialists. The study was approved by Institutional Ethics Committee.

DNA Extraction and Genotyping of XRCC2

Five milliliters of peripheral blood was collected into EDTA-coated tubes and stored at -70°C until analyzed. Genomic DNA was extracted from whole blood using a salting out method [15].
The Allele Refractory Mutation System (ARMS) PCR was used to detect the mutation of interest (AGC>AGG, p.Ser150Arg) in exon 3 of the *XRCC2* gene.
The primers 5' TCAAATACTGCCTGGGAAG 3' and 5' CTGCCATGCCTTACAGAG 3' yielded a 373-bp fragment which were used in combination with ARMS C wild (5' CCTTTTGATTTTGGATAGC 3') and ARMS G mutant (5' TCCAGTAAAAAGCTGACAGC 3') primers, which produced 265-bp and 147-bp fragments, respectively [16]. For each sample, we prepared two PCR reaction tubes with a total volume of 20 µL including 3 µL of genomic DNA including the internal primers and one of the wild or mutant primers for each of them. The PCR program included 30 cycles of initial denaturation at 95°C for 2 minutes followed by cyclic denaturation at 95°C for 45 seconds and annealing at 58°C for 30 seconds and extension at 72°C for 20 seconds. Final extension was set at 72°C for 5 minutes. PCR products were identified on 2% agarose gel. The wild homozygote sample produces a 373 bp and 265 bp band of interest whereas the homozygote mutant one results in a 373 bp and 147 bp band. However, the heterozygote sample yields three bands including 373 bp, 265 bp, and 147 bp.

Statistical analysis

The Chi square (X^2) test was used to verify whether the *XRCC2* genotype distributions were in Hardy-Weinberg equilibrium. Allelic and genotypic frequencies were compared across groups using the X^2 test. The odds ratio (OR) and the corresponding 95% confidence intervals (CIs) between CRC and a detected variant were calculated using logistic regression. All analyses were done with SPSS v 22 software.

RESULTS

A total of 431 samples in two groups, case (patient) and control, were studied. The mean age in patient and control groups was 60.93 and 59.09 years, respectively. The patients comprised 153 males and 138 females (M/F ratio = 1.1) and the control subjects consisted of 67 males and 73 females (M/F ratio = 0.9). No significant gender- or age-related differences were observed between the groups ($p > 0.05$). Furthermore, we did not observe any significant effect of alcohol consumption ($p = 0.77$) and smoking ($p = 0.18$) on susceptibility to colorectal cancer. All demographic data is available in Table 1.
The genotyping results of *XRCC2* c.450C>G were obtained by ARMS PCR and shown in Table 2. Among the colorectal cancer patients, we found the frequency of the *XRCC2* (AGC>AGG, p.Ser150Arg) genotype to be 34.7% for CC, 52.6% for CG and 12.7% for GG, while the frequency in the control population was 97.9% for CC, 2.1% for CG, and 0.0% (non-sample) for GG. The association between the *XRCC2* c.450C>G (p.Ser150Arg) variation and the CRC was found to be significant ($p < 0.001$). In patients, it was found that the *XRCC2* (Serine^{150} Arginine) genotypes with mutant allele (GG + CG) were associated with an increased risk of CRC (OR, 85.9; 95% CI, 26.6 - 276.5; $p < 0.001$).

Table 1. Demographic characteristics and selective risk factors in colorectal cancer cases and controls.

Characteristics	Control (n = 140)	Cases (n = 291)	p-value
Age (mean + SD) years	59.09 ± 12.3	60.93 ± 11.3	0.125
Age group, n (%)			
≤ 50	29 (20.7%)	45 (15.5%)	0.176
> 50	111 (79.3%)	246 (846.5%)	
Gender, n (%)			
Male	67 (47.9%)	153 (52.6%)	0.359
Female	73 (52.1%)	138 (47.4%)	
Alcohol consumption, n (%)			
No	124 (88.6%)	255 (87.6%)	0.778
Yes	16 (11.4%)	36 (12.4)	
Smoking status, n (%)			
No	104 (74.3%)	198 (68%)	0.185
Yes	36 (25.7%)	93 (32%)	

Table 2. Allelic and genotypic frequencies of *XRCC2* AGC>AGG, p.Ser150Arg in cases and controls.

Variable	Cases n (%)	Controls n (%)	OR (95% CI)	p-value	X^2 p-value (overall)
Genotype					
CC	101 (34.7)	137(97.9)	1.00 (ref)	-	< 0.001
CG	153 (52.6)	3 (2.1)	86.6 (25.40 - 295.74)	0.001	
GG	37 (12.7)	0 (0.0)	N/A [a]	< 0.997	
GC/GG	190 (65.3)	3 (2.1)	85.9 (26.68 - 276.54)	< 0.001	
Allele frequency					
C	355 (61.0)	277 (98.9)	59.04 (18.6 - 186)	< 0.001	
G	227 (39.0)	3 (1.1)			

[a] - Not available.

Subsequently, the allele frequency between cases and controls was very different. C Allele had a 61% frequency among cases and 98.9% among controls, and G allele had a 39% frequency in patients and 1.1% among healthy individuals; therefore, the G allele increased the risk of CRC (OR, 59.0; 95% CI, 18.6 - 186; $p < 0.0001$).

Analysis of the *XRCC2* c.450C>G genotypes with that of the demographic and risk parameters also revealed no significant associations with many parameters (Table 3). The *XRCC2* (p.Ser150Arg) variation was only associated significantly with age ($p = 0.017$) in CRC patients.

DISCUSSION

The RAD51-like gene family is composed of XRCC2, XRCC3, RAD51L1, RAD51L2, and RAD51L3 in somatic mammalian cells. There are some indications that XRCC2 has a key role in enhancing the action of RAD5. A previous study demonstrated that the lack of XRCC2 function in cells can decrease RAD51 responses about five-fold. Therefore, XRCC2 is known as a repair response enhancing factor [17].

Although the human *XRCC2* is known as a conserved gene, several *XRCC2* gene polymorphisms have been found. Previously, different *XRCC2* gene polymor-

Table 3. Association between *XRCC2*AGC>AGG, P. Ser150Arg variation and sample characteristics.

Variables			Case (n = 291)					Control (n = 140)		
	n (%)	CC 101 (34.7%)	CG 153 (52.6%)	GG 37 (12.7%)	p-value	n (%)	CC 137 (97.9%)	CG 3 (2.1%)	GG 0	p-value
Age group										
< 50	40 (13.7)	14	18	8	0.295	43 (30.7)	43	0	0	0.244
> 50	251 (86.3)	87	135	29		97 (69.3)	94	3	0	
Gender										
Female	138 (47.4)	59	78	16	0.243	83 (59.3)	80	3	0	0.147
Male	153 (52.6)	42	75	21		57 (40.7)	57	0	0	
Smoking status										
No	198 (68.0)	90	87	21	0.000	104 (74.3)	101	3	0	0.303
Yes	93 (32.0)	11	66	16		36 (25.7)	36	0	0	
Alcohol consumption										
No	255 (87.6)	91	132	32	0.647	124 (88.6)	121	3	0	0.529
Yes	36 (12.4)	10	21	5		16 (11.4)	16	0	0	

phisms have been investigated with regard to susceptibility to different cancers including colorectal cancer. The *XRCC2* polymorphisms commonly studied were C41657T [18], G4234C [18], and Arg188His (rs3218 536) [19,20]. Most of the reported results varied in the different studies implying that further investigations are needed. For instance, Krupa R et al. studied *RAD51*, *XRCC2*, and *XRCC3* polymorphisms in 100 Polish CRC patients [19]. They showed that *XRCC2* Thr 241 Met can increase the risk of CRC when it is in combination with *XRCC3* Arg188 His and also decrease the risk of cancer when it is combined with *RAD51* C135C. Also, in another study which was done in Turkish population, *XRCC2* Arg188His polymorphism was compared between healthy individuals and CRC patients. The results showed that the frequency of XRCC2 polymorphism was two-fold higher in CRC patients than in the healthy group. They suggested that it might be an early diagnostic marker for CRC [20].

Only the study by Fayaz et al. has reported a novel missense variation (AGC>AGG, p.Ser150Arg) in the *XRCC2* gene in one sample of the 50 healthy people analyzed in Iran. However, no alteration was detected in the differentiated thyroid carcinoma (DTC) samples screened for this variation using the ARMS method [16]. Also, this variation presented as a single nucleotide variant in the ClinVar database, but it implies that *XRCC2* AGC>AGG, p.Ser150Arg has uncertain clinical significance.

XRCC2 protein contains two highly conserved ATP binding motifs: Motif A and Motif B. Motif B is followed by Serine ^{150} residues [21]. Previous studies have demonstrated that conservative substitution of the lysine in Motif A resulted in a protein with limited ATP binding activity [22]. Researchers suggested that the loss of a positively charged residue at this position will probably abolish ATP binding function [23,24]. Accordingly, a serine to arginine (p.Ser150Arg) substitution in the motif B nearby residue and charge would alter this position, most probably affecting the ATP binding activity. Consequently, *XRCC2* (p.Ser150Arg) variation probably increased sensitivity to DNA damaging agents and reduced HR repair, leading to cancer development. In regard to this hypothesis, further investigations on the role of *XRCC2* (p.Ser150Arg) variation in cancer development are needed. Therefore, in this attempt we investigated the *XRCC2* (p.Ser150Arg) variation in 140 healthy Iranian people and 291 CRC patients. Our results revealed that the only three healthy people were carriers of the G allele and none were GG homozygous (Table 2). This result reflects the result of Fayaz et al., who realized one sample of 50 control individuals had this altered allele [16]. More interestingly, the number of G alleles was the lowest among the control group (1.1%), whereas it was obviously high among CRC samples (39%). This indicated that the G allele is associated with the development of CRC (OR, 59.0; 95% CI, 18.6 - 186; $p < 0.0001$).

Based on the appropriate sample size in this study in comparison with a previous report [16], we believe that the *XRCC2* (p.Ser150Arg) variation can promote cancers. However, further evaluations on other cancers and populations are recommended.

CONCLUSION

Our pilot study provides evidence that a novel missense variation (AGC>AGG, p.Ser150Arg) in the *XRCC2* gene is associated with development of CRC. Notably, the association was stronger in individuals with mutant allele genotypes. However, these preliminary data require validation in larger independent populations as well as other types of cancers. To our knowledge, this study not only conducted assessment of *XRCC2* AGC>AGG, p.Ser150Arg variation in large number of normal Iranian population, but also evaluated the role of this variation in susceptibility to colorectal cancer for the first time.

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Declaration of Interest:
No conflict of interest to declare.

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