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RESEARCH

Susceptibility to Colorectal Cancer and Two Genetic Polymorphisms of *XRCC4*

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Abstract The X-ray complementing group 4 (XRCC4, OMIM: 194363) plays a key role in non-homologous end-joining DNA repair pathway in mammalian cells. This pathway is believed to help maintain genomic stability. In the present study, it is hypothesized that genetic polymorphisms in the NHEJ repair *XRCC4* gene may be associated with an increased risk in developing colorectal cancer (CRC). We genotyped two polymorphisms of *XRCC4*, G-1394T (rs6869366) and intron 3 insertion/deletion (I/D; rs28360071) in 200 colorectal cancer patients as well as 256 healthy individuals, and evaluated their association with CRC. We found that in G-1394T polymorphism, neither the TG nor the GG genotypes (versus the TT genotype) were associated with the risk of developing CRC. The results of our study indicate that in comparison with the II genotype, ID and DD genotypes had no significant association with the risk of developing CRC. Subjects with TT genotype and positive family history in colorectal cancer were found to be at a much lower risk of developing CRC in comparison with the reference group (OR=0.31, 95%CI: 0.11–0.85, P=0.023). It should be noted that participants having at least one G allele (TG+GG genotypes) were at a significantly higher risk to develop the disease compared with the reference group (OR=9.10, 95%CI: 2.00–41.3, P=0.004). In relation to I/D polymorphism, among participants, those with positive family history, either with ID (OR=4.78, 95%CI: 2.26–10.0, P<0.001) or DD genotypes (OR=5.73, 95%CI: 1.99–16.4, P=0.001) had a significantly association with the disease. Among participants with a positive family history in CRC, the haplotype GD dramatically increased the risk of developing CRC (OR=10.2, 95%CI: 2.28–46, P=0.002). The results of this study indicate that G-1394T and I/D polymorphisms of *XRCC4* among individuals with positive family history for colorectal cancer substantially increase the risk factor for developing colorectal cancers.

Keywords Colorectal cancer · Polymorphisms · *XRCC4*

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Introduction

Colorectal cancer (CRC) is the third most typically diagnosed cancer and the third basic cause of cancer death in the world [1, 2]. Although the etiology of CRC remains largely unknown, several environmental (such as cigarette smoking and exposure to aromatic amines) and genetic factors are known to increase risk of developing CRC [3, 4].

Some of the risk factors associated with the disease can result in DNA damage such as the double strand breaks (DSBs). The DSBs have the most harmful and damaging effect on genome; leading to apoptosis or tumorgenesis [5]. In mammalian, there are two distinct pathways for DNA double strand breaks repair: homologous recombination (HR) and non-homologous end-joining (NHEJ) pathways [6]. The gene encoding X-ray complementing group 4 (*XRCC4*, OMIM: 194363) is one of the genes associated with the NHEJ repair pathway [7]. The XRCC4 protein is believed to help repair the DNA DSBs, supporting V (D) J recombination and always complex with Ligase IV [8–10]. It has been reported that genetic polymorphisms of NHEJ genes influence the DNA repair capability. Further, they have been associated with several type of cancer, including bladder [11], breast [12–14], gastric [15], oral [16] and colorectal [17].

Various recognized polymorphisms of the *XRCC4* gene, including G-1394T (rs6869366) and an insertion/deletion (I/D) in intron 3 (rs28360071) have been the subject of previous research [17]. Therefore, we focused our study on the two polymorphisms of *XRCC4*; hypothesizing polymorphisms in *XRCC4* that are suspected to significantly increase the possibility of genomic instability, leading to colorectal cancer. The current study was conducted to test the merits of this hypothesis.

Materials and Methods

The case-control study consisted of 200 colorectal cancer patients who were referred to the Chemotherapy Department of the Namazi Hospital in the Iranian city of Shiraz and 256 healthy blood donors were also selected as the control group. The control group was frequency-matched with the age and gender of the patients. Because of the fact that Iranian population is one of the most heterogeneous populations in the world [18–20], we selected our patients and the control group from the Persian Muslims living in the Fars Province in Iran. The participants were asked to fill a self-administered questionnaire and to provid peripheral blood samples. Mean (SD) age of the patients and the controls were 54.1 (14.2) and 52.8 (10.7) years, respectively. A questionnaire was administered to the control group and cancer patients, which included questions on familial history of cancers, smoking habits, ethnicity and age at the time of diagnosis. Our study was approved by the Ethics Committee of the Shiraz University and was conducted with prior knowledge of the participants which included a written consent.

Genomic DNA was isolated from EDTA treated blood samples. The genotypes of G-1394T and I/D polymorphisms of *XRCC4* were determined using the PCR based methods with the primers as described in a previous study [17].

In order to make the control group representative of the general population and to eliminate or minimize the possibility of genotyping error, we calculated the Hardy-Weinberg equilibrium using chi-square test. The colorectal cancer risk associated with the genotypes of the study polymorphisms of XRCC4 was estimated using adds ratio (ORs) and 95 % confidence intervals (CIs). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) (version 11.5).

The software SNPAlyze(TM) ver. 6 Standard (Dynacom Co, Ltd. Kanagawa, Japan) was used to evaluate the status of pair wise linkage disequilibrium for the studied polymorphisms. A probability of $P<0.05$ was considered to be statistically significant.

Results

The general characteristics of colorectal cancer patients and control group are summarized in Table 1. Family history significantly differed between cases and controls (OR=3.97, 95 % CI: 2.4–6.57, $P=0.001$). However, smoking habit and consanguinity did not differ significantly between cases and controls ($P>0.05$). The frequencies of the genotypes for the *XRCC4* polymorphisms in the colorectal cancer and control groups are shown in Table 2. The genotypic frequencies of the XRCC4 I/D and G/T polymorphisms in healthy controls (For G-1394T polymorphism: $\chi^2=0.94$, df=1, $P=0.331$; For I/D polymorphism $\chi^2=1.16$, df=1, $P=0.280$) were found to be consistent with the Hardy–Weinberg equilibrium distribution.

For the G-1394T polymorphism, neither the TG (OR=1.02, 95%CI: 0.57–1.82, $P=0.948$) nor the GG (OR=1.04, 95%CI: 0.17–9.21, $P=0.803$) genotypes versus the TT genotype were associated with risk of developing colorectal cancer (Table 2). For the I/D polymorphism, the ID (OR=1.27, 95%CI: 0.84–1.94, $P=0.250$) and DD (OR=1.39, 95%CI: 0.82–2.36, $P=0.211$) genotypes were found to have no significant association with the risk of developing colorectal cancer, in comparison with the II genotype (Table 2).

The participants were stratified by their family history of colorectal cancer (negative *vs* positive) and the data was reanalyzed. Table 2 also shows the profiles of the *XRCC4* polymorphisms and family history for colorectal cancer in the first-degree relatives in the control and cancerous groups. The reference group consisted of individuals with negative family history in colorectal cancer and II genotype (for I/D polymorphism) or TT genotype (for G-1394T polymorphism). Subjects with TT genotype and

Table 1 Comparison of selected risk factors between colorectal cancer patients and healthy control subjects

Variable	Controls	Cases	OR	95 % CI	*P*
Smoking status					
Nonsmoker	196	132	1.0	–	–
Smoker	57	57	1.48	0.97–2.28	0.07
Missing	3	11			
Family history					
Negative	205	133	1.0	–	–
Positive	26	67	3.97	2.4–6.57	0.001
Missing	25	0	–	–	–
Consanguinity					
Negative	170	108	1.0	–	–
Positive	61	37	0.95	0.59–1.53	0.848
Missing	25	55	–	–	–

Table 2 Association between genetic polymorphisms of *XRCC4* and risk of colorectal cancer in relation to their family history of cancer

Family history	Polymorphisms	Controls	Cases	OR	95 % CI	*P*
All	G-1394T					
	TT	225	175	1.0	–	–
	TG	29	23	1.02	0.57–1.82	0.948
	GG	2	2	1.04	0.17–9.21	0.803
	G-1394T					
Negative	TT	182	120	1.0	–	–
	TG	21	12	0.86	0.41–1.82	0.707
	GG	2	1	0.75	0.06–8.45	0.822
Positive	TT	24	55	0.31	0.11–0.85	0.023
	TG	2	11	8.34	1.81–38.2	0.006
	GG	0	1	–	–	–
	TG+GG	2	12	9.10	2.00–41.3	0.004
All	I/D					
	II	95	62	1.0	–	–
	ID	115	96	1.27	0.84–1.94	0.250
	DD	46	42	1.39	0.82–2.36	0.211
	I/D					
Negative	II	78	49	1.0	–	–
	ID	93	60	1.02	0.63–1.66	0.914
	DD	34	24	1.12	0.59–2.11	0.718
Positive	II	9	13	2.29	0.91–5.78	0.077
	ID	12	36	4.78	2.26–10.0	<0.001
	DD	5	18	5.73	1.99–16.4	0.001

positive family history were found to be at a significantly lower risk of developing CRC in comparison with the reference group (OR=0.31, 95%CI: 0.11–0.85, P=0.023). It should be noted that participants having at least one G allele (TG+GG genotypes) were found to be at a significantly higher risk to be patient compared with the reference group (OR=9.10, 95%CI: 2.00–41.3, P=0.004). In relation to the I/D polymorphism, among the participants with a positive family history in CRC, either ID (OR=4.78, 95%CI: 2.26–10.0, P<0.001) or DD genotypes (OR=5.73, 95%CI: 1.99–16.4, P=0.001) had a significant association with the risk of developing colorectal cancer.

The haplotypic prevalence among the participants stratified by the family history of colorectal cancer is shown in Table 3. There were significant differences between frequencies of the haplotypes among the cases and controls (Table 3). Interestingly, among those with a positive family history, the haplotype GD dramatically increased the risk of developing colorectal cancer (OR=10.2, 95%CI: 2.28–46, P=0.002).

Table 3 Haplotype frequencies of *XRCC4* in healthy subjects and colorectal cancer patients in relation to their family history of cancer

Family history	Haplotypes		Controls	Cases	OR	95 % CI	*P*
	G-1394T	I/D					
All	T	I	305	220	1.0	–	–
	T	D	174	153	1.21	0.92–1.61	0.162
	G	D	33	27	1.13	0.66–1.94	0.646
Negative	T	I	249	158	1.0	–	–
	T	D	136	94	1.08	0.78–1.51	0.612
	G	D	25	14	0.88	0.44–1.74	0.720
Positive	T	I	30	62	3.25	2.01–5.26	<0.001
	T	D	20	59	4.64	2.69–8.01	<0.001
	G	D	2	13	10.2	2.28–46.0	0.002

Discussion

To the best of our knowledge, there is just one study investigating the role of *XRCC4* in CRC in the Taiwanese population [17]. Based on that study and the other previous studies, the G-1394T and I/D polymorphisms of *XRCC4* are shown to be related to some cancers. The *XRCC4* G-1394T polymorphism is associated with children leukemia [21], breast [14], gastric [15], prostate [22], bladder [23], lung [24] and colorectal [17] cancers. The *XRCC4* intron3 I/D is also related to oral cancer [16], children leukemia [21] and prostate cancer [25]. However, our results are in contrast with the previous reports in that they show no relationship between these two genetic polymorphisms and the risk of developing CRC.

It has been established that one of the strongest risk factor in developing colorectal cancer is a positive family history in colorectal cancer among the first-degree relatives [26–28]. Surprisingly, in our present study, subjects with the TT genotype and a positive family history in colorectal cancer were at a significantly lower risk of developing CRC, in comparison with the reference group. However, it should be noted that according to the other published research [17], the participants having at least one G allele (TG+GG genotypes) were at a significantly higher risk compared with the reference group. In relation to the I/D polymorphism, the participants with a positive family history in colorectal cancer, either the ID or DD genotypes showed a significant association with the risk of developing colorectal cancer.

The results of our research indicate that the G-1394T and I/D polymorphisms of *XRCC4* among individuals with a positive family history of colorectal cancer can lead to an increased risk for developing colorectal cancer. It should be noted that because of the small size of the population under study, dividing the participants according to their family history of colorectal cancer may have lead to a false positive due to population stratification. This is because larger populations in risk-factor stratification are expected to yield a better understanding of the genetic relations of specific genes with colorectal cancer. Previously, it has been reported that ethnicity may influence the observed associations in multifactorial disease [29–31]. Therefore, replication of the present study in other countries is recommended. Such studies may reveal an alternate method of evaluating the risk of developing CRC in individuals with a positive family history by combining the, two polymorphisms G-1394T and ID of *XRCC4* with positive family history as a risk factor. This in turn, is expected to improve the existing the preventive care measures for this disease.

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Conflict of Interest The authors have no conflict of interest in relation to this study.

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