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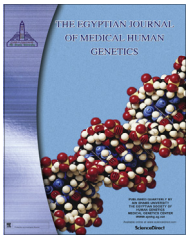

ORIGINAL ARTICLE

DNA repair gene *XRCC7* G6721T variant and susceptibility to colorectal cancer

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KEYWORDS

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Abstract *Background:* The human *XRCC7* (MIM: 600899) is a DNA double-strand break repair gene, involved in non-homologous end joining (NHEJ). Polymorphism G6721T (rs7003908) is located in the intron 8 of the *XRCC7*. This polymorphism may regulate splicing and cause mRNA instability.

Aim: The aim of the present study was to determine an association of G6721T *XRCC7* polymorphism in colorectal cancer.

Subjects and methods: The study included 166 patients with colorectal cancer and 260 age and gender frequency-matched controls. The patients and controls were Iranian (Caucasian/Muslims).

Results: Our data did not demonstrate any statistically significant association between the genotypes of *XRCC7* G6721T polymorphism and risk of colorectal cancer. There was a significant association between family history of cancers among their first-degree relatives (FH) and risk of colorectal cancer (OR = 3.69, 95% CI: 2.19–6.23, $P < 0.001$). We further analyzed to see if the FH influenced the association of the *XRCC7* G6721T polymorphism and colorectal cancer risk. The TT genotype among positive FH persons, remarkably increased the risk of colorectal cancer (OR = 6.88, 95% CI: 2.27–20.8, $P = 0.001$).

Conclusion: The present study suggests the TT genotype of the *XRCC7* G6721T polymorphism might be a risk factor for the development of colorectal cancer among persons with positive FH.

1. Introduction

DNA double-strand breaks (DSBs) may result in genetic instability and ultimately may be associated with a higher rate of cancer development. There are two pathways for DSBs repair, homologous recombination (HR) and non-homologous end joining (NHEJ) pathways. In NHEJ, the broken DNA termini are first processed to make them compatible and then sealed by a ligation step. The NHEJ is an error-prone pathway [1].

The human X-ray cross-complementing group 7 (*XRCC7*; MIM: 600899; GenBank accession no: NM_001469) is a NHEJ repair gene, which encodes the catalytic subunit of DNA-activated protein kinase (DNA-PKcs) [1]. DNA-PKcs is recruited to the site of DSBs by the Ku70/Ku80 heterodimer to form an active DNA-PK complex that is essential for the progression of the pathway [2]. Mice with inactivated compo-

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nents of DNA-PK show severe combined immunodeficiency as well as ionizing radiation hypersensitivity [3,4].

Genetic variation G6721T (rs7003908) is located in the intron 8 of the *XRCC7* gene. This polymorphism may regulate splicing and cause mRNA instability [2]. Although NHEJ is the major pathway for DSB repair in human cells and acts during all phases of the cell cycle [1], only a few studies have been reported on the association between G6721T polymorphism of the *XRCC7* gene and risk of multifactorial traits including, several types of cancers. However the results were not consistent [5–17].

Colorectal cancer is a multifactorial trait. Many genetic variations and epigenetic modifications are involved in the pathology of colorectal cancer [18]. To the best of our knowledge, there is no report on the association between this polymorphism and susceptibility to colorectal cancer. Therefore the present study was carried out.

2. Subjects and methods

2.1. Subjects

The present case-control study consisted of 166 patients (63 females, 103 males) with pathologically confirmed primary colorectal cancer who were recruited from chemotherapy department of Nemazi hospital in Shiraz (south of Iran). Age and gender frequency-matched controls (260 persons; 76 females, 184 males) were randomly selected from the healthy blood donors. The mean age (SD; Min–Max) of the patients and the controls were 53.0 (10.8; 21–85) and 54.0 (14.4; 15–81) years, respectively. Control subjects had a negative history for the diagnosis of any type of cancers. Considering that Iranian population is one of the most heterogeneous populations [19–21], we select our patients and controls from Persian (Caucasians) Muslims living in Fars province.

The Local Ethic Committee approved the study, and each patient gave a written consent. The work has been carried out in accordance with the Code of Ethics of the world Medical Association (Declaration of Helsinki) for experiments in humans.

2.2. DNA extraction and genotyping analysis

Genomic DNA was extracted from whole blood samples. The detection of the *XRCC7* G6721T (rs7003908) polymorphism was carried out using PCR–RFLP technique, as described previously [5]. A negative control containing all reagents but water instead of the DNA template was included in each amplification set. Any sample with ambiguous result due to low yield was retested and a random selection of 15% of all samples was repeated. No discrepancies were discovered upon replicate testing.

2.3. Statistical analysis

For the *XRCC7* G6721T polymorphism, deviation of the genotypic frequencies in the control group from those expected under Hardy–Weinberg equilibrium was assessed using the standard chi squared test. The genotypic-specific risks were estimated as odds ratios (ORs) with associated 95% intervals (CIs) by unconditional logistic regression. In the analysis, the persons with the TT genotype were assumed as reference group.

Positive family history of cancers in first-degree relatives (FH) is one of the strongest risk factor [22–24]. A person with at least one first-degree relative with a kind of cancer was considered to have a positive family FH. Therefore, the participants were stratified by their FH (negative and positive) and the data were reanalyzed. For these analyses, persons who have negative FH were assumed as reference group. Data on FH in the control subjects were missed for some participants. It should be noted that among control subjects of the present study, 11.2% had positive FH (see Section 3). In order to study the potential effect of FH on colorectal cancer risk as well as the risk associated with genotypes of the study polymorphism, the "sensitivity analysis" was used. For this analysis we tested two assumptions for the missing data: all the missed data had negative FH (assumption I); and alternatively, 25% of them had positive (and 75% had negative) FH (assumption II).

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) (version 11.5). A probability of $P < 0.05$ was considered statistically significant. All P values were two-tailed.

3. Results and discussion

The control and patient groups were initially divided into two gender groups. Since no statistical differences were observed between genders for the frequencies of genotypes of *XRCC7* G6721T polymorphism, the gender groups were pooled (for control group: $\chi^2 = 1.27$, df = 2, $P = 0.530$; for patient group: $\chi^2 = 1.54$, df = 2, $P = 0.463$).

Table 1 shows the genotypic distribution of the *XRCC7* G6721T polymorphism between the patients and their controls. The observed genotypic frequency in the control group was in agreement with Hardy–Weinberg equilibrium ($\chi^2 = 1.22$, df = 1, $P = 0.268$). As shown in Table 1, our data did not demonstrate any statistically significant association between the genotypes and risk of colorectal cancer (for TG vs. TT: OR = 1.20, 95% CI: 0.75–1.93, $P = 0.434$; for GG vs. TT: OR = 1.05, 95% CI: 0.61–1.81, $P = 0.582$).

A few association studies have been published which investigated the relationship between *XRCC7* G6721T polymorphism and risk of cancers (such as bladder cancer, prostate cancer and renal cell carcinoma) [5–14]. The majority of these studies found no association between this polymorphism and risk of cancers, which is very similar to our finding.

Several studies revealed that positive FH is one of the strongest risk factors for cancers [22–24]. The prevalence of positive FH among controls and patients were 11.2% and 31.9%,

Table 1 Association between *XRCC7* polymorphism and susceptibility to colorectal cancer.

Genotypes	Patients	Controls	OR	95% CI	*P*-value
TT	42	73	1.0	–	–
TG	84	121	1.20	0.75–1.93	0.434
GG	40	66	1.05	0.61–1.81	0.852
TG + GG	124	187	1.15	0.74–1.79	0.529

Table 2 Association between *XRCC7* polymorphism and risk of colorectal cancer after stratification of participants according to family history of cancers in their first-degree relatives.

Genotypes	Family history	Patients (*n*)	Control (*n*)	OR	95% CI	*P*
TT	Negative	27	62	1.0	–	–
TT	Positive	15	5	6.88	2.27–20.8	0.001
TG + GG	Negative	86	143	1.0	–	–
TG + GG	Positive	38	21	3.01	1.65–5.46	<0.001

Table 3 Sensitivity analysis for association between *XRCC7* polymorphism and risk of colorectal cancer under our two assumptions for missing data in control group.

Genotypes	Family history	Assumption I			Assumption II		
		OR	95% CI	*P*-value	OR	95% CI	*P*-value
TT	Negative	1.0	–	–	1.0	–	–
TT	Positive	7.55	2.50–22.8	<0.001	6.20	2.17–17.6	0.001
TG + GG	Negative	1.0	–	–	1.0	–	–
TG + GG	Positive	3.49	1.93–6.32	<0.001	2.61	1.49–4.57	0.001

Notes: under assumption I and assumption II we assumed that all the missed data had negative family history; and 25% of them had positive (and 75% had negative) family history, respectively.

respectively. Therefore, there was a significant association between FH and risk of colorectal cancer (OR = 3.69, 95% CI: 2.19–6.23, $P < 0.001$). We further analyzed to see if FH influenced the association of the polymorphism and colorectal cancer risk (Table 2). The TT genotype among positive FH persons, remarkably increased the risk of colorectal cancer (OR = 6.88, 95% CI: 2.27–20.8, $P = 0.001$). In the TG + GG genotypes, the risk of colorectal cancer increased 3.01-fold among positive FH persons (Table 2), which it is very similar to the estimated risk of positive FH (OR = 3.69). Taken together, it seems that these genotypes did not alter the risk of colorectal cancer.

It should be noted that the main finding of the present study has some limitations. Data on the FH missed for 29 participants in control group. In order to show that the observed associations did not occur due to the maldistribution of the genotypes among missing data, sensitivity analysis was performed. Sensitivity test was carried out under our two assumptions as mentioned in Section 2.3. Table 3 shows the results of statistical analysis under our assumptions. We found that our main finding is not a false finding due to maldistribution of missing data. Taken together, the TT genotype among positive FH persons remarkably increased the risk of colorectal cancer. Very recently, similar to our present finding, it is reported that polymorphisms of the *XRCC4* and *XRCC5* might be risk factors for gastric cancer development especially among persons with positive FH [25]. Also it has been reported that *XRCC7* G6721T polymorphism had no effect on hepatocellular carcinoma risk to the whole population, but had a protective effect on HCC risk among males and alcohol drinkers [17].

Although the functional relevance of the *XRCC7* polymorphism is unknown, several lines of evidence suggest that our finding is biologically plausible. The *XRCC7* gene is located on chromosome 8q11 and encodes the DNA-PKcs, which plays a key role in NHEJ pathway for DSBs [26]. It has been suggested that human chromosome 8q11 functionally corrects the hyper-radiosensitivity and variable (diversity) joining region recombination in severe combined immunodeficiency cells and complements the DSBs repair deficiency of severe combined immunodeficiency cells that are phenotypically sensitive to radiation-induced chromosome aberration [26–28]. Although the functional significance of the G6721T *XRCC7* polymorphism is unknown, this intronic genetic variation might regulate splicing and cause mRNA instability [2] or may be a haplotype with other genetic changes in other disease-related genes through a linkage disequilibrium mechanism [29]. However, these possibilities should be investigated in future studies.

It has been reported that there is significant difference between ethnicity and susceptibility to cancers in relation to other polymorphisms [30–32]. Therefore, larger studies with detailed data on environmental exposure from different ethnic groups are needed to verify this initial finding.

Conflict of interest

Authors have no financial or any non-financial competing interests.

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