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RESEARCH

Susceptibility to Gastric Cancer and Polymorphisms of Insertion/Deletion at the Intron 3 of the *XRCC4* and VNTR at the Promoter Region of the *XRCC5*

Mostafa Saadat · Samira Pashaei · Foroozan Amerizade

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Abstract The genes encoding X-ray repair cross-complementing group 4 (*XRCC4*; OMIM: 194363) and 5 (*XRCC5*; OMIM: 194364) are involved in repair of DNA double-strand breaks. To investigating the associations between polymorphisms of Insertion/Deletion (I/D, rs28360071) in the intron 3 of the *XRCC4* and VNTR in the promoter region of the *XRCC5* and risk of gastric cancer, the present study was carried out. We included 159 (56 females, 103 males) with gastric cancer and 242 (75 females, 167 males) healthy blood donors frequency matched for age and gender. Using PCR-based methods, the genotypes of the study polymorphisms were determined. The alleles of VNTR *XRCC5* polymorphism divided into two groups: L (0 and 1 repeats) and H (2 and 3 repeats) alleles. For the I/D *XRCC4* polymorphism, after stratification of the subjects according to their family history (FH) of cancer, either the ID (OR=3.19, 95%CI: 1.35–7.50, *P*=0.008) or the DD genotypes (OR=4.62, 95%CI: 1.63–13.0, *P*=0.004) among positive FH persons, increased the risk of gastric cancer compared with the reference group (persons who have negative FH and II genotype). For the VNTR *XRCC5* polymorphism, the LH+HH genotypes among positive FH persons, increased the risk of gastric cancer compared with the reference group (persons who have negative FH and LL genotype) (OR=2.88, 95%CI: 1.34–6.18, *P*=0.006). Sensitivity analysis showed that the above mentioned associations were not occurred due to the maldistribution of the genotypes among missing data. The present study suggests that both polymorphisms of the *XRCC4* and *XRCC5* might be risk factors for gastric cancer development especially among persons with positive FH.

Keywords Gastric cancer · Polymorphism · Ins/Del · VNTR · *XRCC4* · *XRCC5*

Introduction

Gastric cancer is a major health problem worldwide due to its prevalence, poor prognosis and limited treatment options. It is well established that both environmental and genetic factors involved in the development of gastric cancer [1, 2].

DNA double-strand breaks (DSBs) occur spontaneously during the cell cycle and are induced by a variety of exogenous agents. DSBs are major lesions that destroy the integrity of the DNA molecule. To combat this potentially lethal damage, two related repair pathways, namely homologous recombination (HR) and non-homologous DNA end joining (NHEJ), have been evolved. The HR is a template guided and error-free pathway. In most cases, the NHEJ pathway results in the loss of a few nucleotides at the broken ends, making this pathway error-prone. Unrepaired DSBs may result in genetic instability and ultimately may enhance the rate of cancer development. It have been reported that NHEJ deficiencies can lead to increased genomic instability and cause increased tumorigenesis [3–8]. Ku70 and Ku80 form a Ku complex which is a heterodimeric DNA binding complex involved in the repair of DSBs as a member of the NHEJ pathway. Ku80 is encoded by the X-ray repair cross-complementing group 5 gene (*XRCC5*; OMIM: 194364) [9]. It has been reported that mouse cells deficient for Ku80 display a marked increase in chromosomal aberrations,

Samira Pashaei and Foroozan Amerizade Have equal contributions.

M. Saadat (✉) · S. Pashaei · F. Amerizade
Department of Biology, College of Sciences, Shiraz University, Shiraz 71454, Iran
e-mail: saadat@shirazu.ac.ir

M. Saadat
e-mail: msaadat41@yahoo.com

M. Saadat · S. Pashaei · F. Amerizade
Institute of Biotechnology, Shiraz University, Shiraz 71454, Iran

including breakage, translocations, and aneuploidy [4]. It has been well shown that the X-ray repair cross-complementing group 4 gene (*XRCC4*; OMIM: 194363) plays important role in repair of DSBs [10]. Deficiency of XRCC4 in primary murine cells causes growth defects, premature senescence, IR sensitivity, and inability to support V(D)J recombination have been reported [11].

Several genetic polymorphisms in the *XRCC4* and *XRCC5* have been reported in human. An Insertion/Deletion (I/D) of a 30 bp sequence in intron 3 of the *XRCC4* gene (rs28360071) has been reported. Associations between the I/D polymorphism of *XRCC4* and susceptibility to several types of cancers, including childhood leukemia, prostate, oral and colorectal cancers were reported [12–15]. A variable number of tandem repeats of a 21 bp (VNTR) polymorphism at the promoter region of the *XRCC5* gene have been reported [9]. This polymorphism has four alleles: 3R, 2R, 1R and 0R [16]. It is established that the mRNA level of XRCC5 decreased as function of number of tandem repeats at the promoter region of the gene [17]. Considering that environmental factors induced DNA damage and on the other hand, functional polymorphisms in the DNA repair genes may alter the cellular DNA repair capacity; therefore, study of association between genetic variations in DNA repair genes and risk of gastric cancer is potentially important. It should be noted that the over expression of the *XRCC5* in several types of cancers (such as gastric and colorectal cancers) has been reported [18–20]. Take together, it may be hypothesized that the 3R and 2R (High repeated) alleles compared to 0R and 1R (Low repeated) alleles alter the risk of development of cancers. There is no so much data on associations between polymorphisms of the *XRCC5* and susceptibility to cancers [21–26]. However, there is no study investigating the association between VNTR polymorphism at the promoter region of the *XRCC5* and I/D polymorphism of *XRCC4* and susceptibility to gastric cancer. Therefore, the present case-control study was carried out.

Materials and Methods

Subjects

The present case-control study was consisted of 159 (56 females, 103 males) with pathologically confirmed primary gastric cancer that were recruited from chemotherapy department of Nemazi hospital in Shiraz (Fars province, south of Iran). Age and gender frequency-matched controls were randomly selected from the healthy blood donors. A total of 242 (75 females, 167 males) healthy controls was included in the study. Exclusion criteria for controls included any previous cancer history and diagnosed psychiatric diseases. The mean age (SD; Min-Max) of the patients and the controls were 57.3 (12.9; 24–85) and 56.7 (9.8; 31–82)years, respectively. There was no significant difference between cases and controls for age of subjects ($P>0.05$). The Iranian population is one of the most heterogeneous populations [27, 28]. Therefore, we selected our patients and controls from Persian (Caucasian) Muslims living in Fars province (southern Iran). Information on family history of cancer was collected for all participants via in-person interviews. A person with at least one first-degree relative with diagnosed cancer is considered to have a positive family history. Informed consent was obtained from each subject before the study. Ethical approval for the current study was obtained from Shiraz University institutional review board.

DNA Extraction and Genotyping Analysis

Blood samples were collected from the subjects. Genomic DNA was extracted from whole blood samples. Genotypic analysis for the I/D polymorphism of the *XRCC4* was determined by PCR assay, as described previously [12]. PCR amplification was performed using following primers: forward primer 5'-TCC TGT TAC CAT TTC AGT GTT AT-3' and reverse primer 5'-CAC CTG TGT TCA ATT CCA GCT T-3'. The genotypic analysis for the VNTR polymorphism of *XRCC5* was determined by PCR assay, as described previously [29]. PCR amplification was performed using following primers: forward primer 5'-AGG CGG CTC AAA CAC CAC AC-3' and reverse primer 5'-CAA GCG GCA GAT AGC GGA AAG-3'. The VNTR alleles divided into two groups: L (means low repeated alleles; 0 and 1 repeat) and H (means high repeated alleles; 2 and 3 repeats) alleles. For quality control, 15 % of randomly selected samples were repeated to verify genotyping results and 100 % concordance was found.

Statistical Analysis

A Chi-square test was performed for the *XRCC5* VNTR and *XRCC4* I/D polymorphisms to determine if the control group demonstrated Hardy-Weinberg equilibrium. The associations between the genotypes of the study polymorphisms and gastric cancer risk were assessed by calculating odds ratios (ORs) and 95 % confidence intervals (CIs). In the analysis, the persons with LL and II genotypes assumed as reference groups, for VNTR *XRCC5* and I/D *XRCC4* polymorphisms, respectively.

Positive family history of cancer in first-degree relatives is one of the strongest risk factor [1, 2, 30–32]. Therefore, the participants were stratified by their family history of cancers (negative and positive) and the data were reanalyzed. For these analyses, persons who have negative family history and the reference genotypes (as mentioned above) assumed as reference groups.

Data on family history in the control subjects were missed for some participants. In order to study the potential effect of

the family history on gastric cancer risk as well as the risk associated with genotypes of the study polymorphisms, the "sensitivity analysis" was used. For this analysis we tested two assumptions for the missing data of the family history in the control group: all the missed data had negative family history; and alternatively, 25 % of them had positive (and 75 % had negative) family history.

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 statistical tests are two-sided.

Results and Discussion

Detailed genotype distributions among cases and controls are summarized in Table 1. The genotypic frequencies of the VNTR *XRCC5* ($\chi^2=4.90$, df=6, $P=0.559$) and I/D *XRCC4* ($\chi^2=0.032$, df=1, $P=0.857$) polymorphisms did not show significant deviation from Hardy-Weinberg equilibrium in control subjects.

Table 2 shows the association between the *XRCC4* polymorphism and susceptibility to gastric cancer. For I/D polymorphism of *XRCC4*, although there was no significant association between the ID genotype and the risk of gastric cancer (OR=1.34, 95 % CI: 0.84–2.15, $P=0.215$), the DD genotype increased the risk of gastric cancer (OR=1.85, 95 % CI: 1.04–3.29, $P=0.034$) (Table 2). The risk of gastric cancer increased as a function of number of the D allele ($\chi^2=4.54$, $P=0.033$). Table 2, also shows the association between the VNTR *XRCC5* polymorphism and susceptibility to gastric cancer. Neither the LH (OR=1.03, 95 % CI: 0.66–1.62, $P=0.882$) nor the HH (OR=0.90, 95 % CI: 0.50–1.61, $P=0.735$) genotypes altered the risk of gastric cancer in comparison with the LL genotype (Table 2).

Table 1 Genotypic prevalence of the VNTR polymorphism at promoter region of the *XRCC5* among gastric cancer patients and healthy control subjects

Genotypes	Cases	Controls
0R0R	2	1
0R1R	13	14
0R2R	10	7
0R3R	0	1
1R1R	39	67
1R2R	66	101
1R3R	1	4
2R2R	27	46
2R3R	1	1

Several studies revealed that positive family history of cancer in first-degree relatives is one of the strongest risk factor for cancers, including gastric cancer [30–32]. The prevalence of positive family history among controls and cases were 14.0 and 28.4 %, respectively. Therefore there was significant association between family history and risk of gastric cancer (OR=2.43, 95 % CI: 1.41–4.20, $P=0.001$). We further analyzed to see if the family history of cancer influenced the association of the *XRCC4* I/D polymorphism and risk of gastric cancer. After stratification of the subjects according to their family history of cancer, either the ID (OR=3.19, 95 % CI: 1.35–7.50, $P=0.008$) or the DD genotypes (OR=4.62, 95 % CI: 1.63–13.0, $P=0.004$) among positive family history persons, increased the risk of gastric cancer compared with the reference group. It should be noted that there was no association between the genotypes of I/D polymorphism and risk of gastric cancer among negative family history persons (Table 2).

For the VNTR *XRCC5* polymorphism, and after stratification of the subjects according to their family history of cancer, the LH + HH genotypes among positive family history persons, increased the risk of gastric cancer compared with the reference group (OR=2.88, 95 % CI: 1.34–6.18, $P=0.006$). It should be noted that there was no association between the genotypes of VNTR polymorphism and risk of gastric cancer among negative family history persons (Table 2).

The results of the present study have some limitations. Data on the family history were missing for 64 participants in control group. Using "sensitive analysis" it is possible to estimate the potential effect of this variable on the study by assuming various degrees of maldistribution of the variable in the control group and seeing how it would affect the results. As mentioned in "Statistical analysis" section, we tested two distributions for the missing data among controls. Table 3 shows the statistical analysis under our two assumptions. Therefore the present case-control study suggests that the study polymorphisms of the *XRCC4* and *XRCC5* genes might be risk factors for gastric cancer development among persons with positive family for cancer. Also we found that these associations are not false findings due to maldistribution of missing data.

The similar association between other polymorphism of *XRCC5* (C74468A) and alter gastric and esophageal cancers were reported [23]. Dong et al. [23] found that in subjects with a familial history of gastric cancer, the C allele of *XRCC5* C74468A seemed to be a protective factor for the incidence. A similar trend was found in the case of esophageal cancer [23].

The influence of 2R and 3R alleles on suppressed expression of the *XRCC5* [17] might confer risk to DNA repair leading to genomic instability and gastric cancer. Hence, the VNTR polymorphism in the promoter region of *XRCC5* gene could serve as an important prognostic marker in gastric cancer development among persons who have positive family history.

Table 2 Associations between the I/D *XRCC4* and VNTR *XRCC5* polymorphisms and risk of gastric cancer

Family history	Genotypes	Cases	Controls	OR	95 % CI	*P*-value
I/D *XRCC4* polymorphism						
All	**II**	42	84	1.0	–	–
	ID	78	116	1.34	0.84–2.15	0.215
	DD	39	42	1.85	1.04–3.29	0.034
Negative	**II**	33	56	1.0	–	–
	ID	56	74	1.27	0.74–2.20	0.376
	DD	24	23	1.84	0.91–3.74	0.088
Positive	**II**	9	9	2.08	0.73–5.89	0.169
	ID	19	12	3.19	1.35–7.50	0.008
	DD	15	4	4.62	1.63–13.0	0.004
	ID+DD	34	16	3.69	1.80–7.59	<0.001
VNTR *XRCC5* polymorphism						
All	**LL**	54	82	1.0	–	–
	LH	77	113	1.03	0.66–1.62	0.882
	HH	28	47	0.90	0.50–1.61	0.735
Negative	**LL**	38	53	1.0	–	–
	LH	54	77	0.97	0.56–1.68	0.936
	HH	21	36	0.81	0.41–1.60	0.552
Positive	**LL**	14	11	1.77	0.72–4.33	0.208
	LH	23	11	2.91	1.27–6.69	0.012
	HH	6	3	2.78	0.65–11.8	0.165
	LH+HH	29	14	2.88	1.34–6.18	0.006

Table 3 Association between genetic polymorphisms of I/D *XRCC4* and VNTR *XRCC5* and risk of gastric cancer under two assumptions for missing data in control group

Family history	Genotypes	Assumption I			Assumption II		
		OR	95 % CI	*P*-value	OR	95 % CI	*P*-value
XRCC4 polymorphism							
Negative	**II**	1.0	–	–	1.0	–	–
	ID	1.22	0.72–2.06	0.440	1.26	0.74–2.16	0.386
	DD	1.53	0.79–2.96	0.202	1.75	0.87–3.49	0.112
Positive	**II**	2.59	0.91–7.30	0.072	2.21	0.78–6.27	0.134
	ID	3.97	1.70–9.28	0.001	3.40	1.45–7.98	0.005
	DD	5.75	2.05–16.1	0.001	4.92	1.74–13.8	0.003
	ID+DD	4.60	2.26–9.31	<0.001	3.94	1.92–8.07	<0.001
XRCC5 polymorphism							
Negative	**LL**	1.0	–	–	1.0	–	–
	LH	0.96	0.59–1.65	0.967	0.98	0.57–1.67	0.944
	HH	0.89	0.46–1.71	0.731	0.84	0.43–1.65	0.619
Positive	**LL**	2.37	0.98–5.74	0.054	1.94	0.79–4.72	0.143
	LH	3.90	1.72–8.86	0.001	3.19	1.39–7.29	0.006
	HH	3.73	0.88–15.7	0.073	3.05	0.72–12.9	0.130
	LH+HH	3.87	1.82–8.19	<0.001	3.16	1.48–6.74	0.003

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

It is also important to keep a number of limitations in mind in considering the present findings. It should be mentioned that the small sample size and thus the limited statistical power to detect differences between the case and control groups, especially when multiple testing was done, is the main limitation of our study. In future, our findings should be confirmed by large-scale studies.

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Conflict of Interest The authors report no conflicts of interest.

References

1. Gao S, Zhang X, Wang P, Dai L, Zhang J, Wang K (2011) Genetic epidemiological analysis reveals a multi-gene additive model for gastric cancer. Fam Cancer 10:119–125
2. Drăghicescu T, Roman IC, Păltănea L (1998) Epidemiologic research on the genetic risk factors in gastric and colorectal cancer. Rom J Morphol Embryol 44:149–152
3. Gilley D, Tanaka H, Hande MP, Kurimasa A, Li GC, Oshimura M, Chen DJ (2001) DNA-PKcs is critical for telomere capping. Proc Natl Acad Sci U S A 98:15084–15088
4. Difilippantonio MJ, Zhu J, Chen HT, Meffre E, Nussenzweig MC, Max EE et al (2000) DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. Nature 404:510–514
5. Ferguson DO, Alt FW (2001) DNA double strand break repair and chromosomal translocation: lessons from animal models. Oncogene 20:5572–5579
6. Gao Y, Ferguson DO, Xie W, Manis JP, Sekiguchi J, Frank KM et al (2000) Interplay of p53 and DNA-repair protein *XRCC4* in tumorigenesis, genomic stability and development. Nature 404:897–900
7. Zhu C, Mills KD, Ferguson DO, Lee C, Manis J, Fleming J et al (2002) Unrepaired DNA breaks in p53-deficient cells lead to oncogenic gene amplification subsequent to translocations. Cell 109:811–821
8. Pfeiffer P, Goedecke W, Kuhfittig-Kulle S, Obe G (2004) Pathways of DNA double-strand break repair and their impact on the prevention and formation of chromosomal aberrations. Cytogenet Genome Res 104:7–13
9. Kuschel B, Auranen A, McBride S, Novik KL, Antoniou A, Lipscombe JM et al (2002) Variants in DNA double-strand break repair genes and breast cancer susceptibility. Hum Mol Genet 11: 1399–1407
10. Li Z, Otevrel T, Gao Y, Cheng HL, Seed B, Stamato TD et al (1995) The *XRCC4* gene encodes a novel protein involved in DNA double-strand break repair and V(D)J recombination. Cell 83:1079–1089
11. Gao Y, Sun Y, Frank KM, Dikkes P, Fujiwara Y, Seidl KJ et al (1998) A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. Cell 95:891–902
12. Chang CH, Chiu CF, Wu HC, Tseng HC, Wang CH, Lin CC et al (2008) Significant association of *XRCC4* single nucleotide polymorphisms with prostate cancer susceptibility in Taiwanese males. Mol Med Rep 1:525–530
13. Mandal RK, Singh V, Kapoor R, Mittal RD (2011) Do polymorphisms in *XRCC4* influence prostate cancer susceptibility in North Indian population? Biomarkers 16:236–242
14. Chiu CF, Tsai MH, Tseng HC, Wang CL, Wang CH, Wu CN et al (2008) A novel single nucleotide polymorphism in *XRCC4* gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol 44:898–902
15. Wu KH, Wang CH, Yang YL, Peng CT, Lin WD, Tsai FJ et al (2010) Significant association of *XRCC4* single nucleotide polymorphisms with childhood leukemia in Taiwan. Anticancer Res 30:529–533
16. Rajaei M, Saadat I, Saadat M (2012) Introducing a novel allele for the polymorphism of variable number of tandem repeats in the promoter region of *XRCC5*. Biochem Biophys Res Commun 427:503–505
17. Rajaei M, Saadat I, Saadat M (2013) The novel allele (3R) of the VNTR polymorphism in the *XRCC5* promoter region dramatically decreases the gene expression. Biochem Biophys Res Commun 430: 640–641
18. Lim JW, Kim H, Kim KH (2002) Expression of Ku70 and Ku80 mediated by NF-kappa B and cyclooxygenase-2 is related to proliferation of human gastric cancer cells. J Biol Chem 277:46093–46100
19. Hosoi Y, Watanabe T, Nakagawa K, Matsumoto Y, Enomoto A, Morita A et al (2004) Up-regulation of DNA-dependent protein kinase activity and Sp1 in colorectal cancer. Int J Oncol 25:461–468
20. Chang HW, Kim SY, Yi SL, Son SH, Song do Y, Moon SY et al (2006) Expression of Ku80 correlates with sensitivities to radiation in cancer cell lines of the head and neck. Oral Oncol 42:979–986
21. Gorre M, Mohandas PE, Kagita S, Annamaneni S, Digumarti R, Satti V (2014) Association of *XRCC5* VNTR polymorphism with the development of chronic myeloid leukemia. Tumour Biol 35:923–927
22. Wang S, Wang M, Yin S, Fu G, Li C, Chen R et al (2008) A novel variable number of tandem repeats (VNTR) polymorphism containing Sp1 binding elements in the promoter of *XRCC5* is a risk factor for human bladder cancer. Mutat Res 638:26–36
23. Dong XJ, Wang N, Guo W, Zhou RM, Zhang XJ, Li Y (2007) Correlations of *XRCC5* polymorphisms to genetic susceptibility to esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma in a high incidence region. Ai Zhengzhou 26:280–284 [Chinese]
24. Li R, Yang Y, An Y, Zhou Y, Liu Y, Yu Q et al (2011) Genetic polymorphisms in DNA double-strand break repair genes *XRCC5*, *XRCC6* and susceptibility to hepatocellular carcinoma. Carcinogenesis 32:530–536
25. Yang MD, Tsai CW, Chang WS, Tsou YA, Wu CN, Bau DT (2011) Predictive role of *XRCC5*/*XRCC6* genotypes in digestive system cancers. World J Gastrointest Oncol 3:175–181
26. Rajaei M, Saadat I, Omidvari S, Saadat M (2014) Association between polymorphisms at promoters of *XRCC5* and *XRCC6* genes and risk of breast cancer. Med Oncol 31:885
27. Rafiee L, Saadat I, Saadat M (2010) Glutathione S-transferase genetic polymorphisms (*GSTM1*, *GSTT1* and *GSTO2*) in three Iranian populations. Mol Biol Rep 37:155–158
28. Mohamadynejad P, Saadat M (2008) Genetic polymorphisms of *XRCC1* (at codons 194 and 399) in Shiraz population (Fars province, southern Iran). Mol Biol Rep 35:669–672
29. Rajaei M, Saadat I, Saadat M (2012) High resolution melting analysis for detection of variable number of tandem repeats polymorphism of *XRCC5*. Biochem Biophys Res Commun 425:398–400
30. Liu J, Shu T, Chang S, Sun P, Zhu H, Li H (2014) Risk of malignancy associated with a maternal family history of cancer. Asian Pac J Cancer Prev 15:2039–2044
31. Jiang X, Tseng CC, Bernstein L, Wu AH (2014) Family history of cancer and gastroesophageal disorders and risk of esophageal and gastric adenocarcinomas: a case-control study. BMC Cancer 14:60
32. Turati F, Negri E, La Vecchia C (2014) Family history and the risk of cancer: genetic factors influencing multiple cancer sites. Expert Rev Anticancer Ther 14:1–4