



Investigating the association between *XRCC1* gene polymorphisms and susceptibility to gastric cancer

S. Chen, X.C. Zhu, Y.L. Liu, C. Wang and K.G. Zhang

Department of Gastroenterology, Anhui Provincial Hospital, Hefei, China

Corresponding author: S. Chen
E-mail: sichensc11@163.com

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ABSTRACT. We carried out a hospital-based case-control study to investigate the role of *XRCC1* gene Arg399Gln, Arg280His, and Arg194Trp polymorphisms in susceptibility to gastric cancer. A total of 214 gastric cancer patients and 247 control subjects were recruited between March 2013 and March 2015, and polymorphism genotype frequencies were determined by polymerase chain reaction-restriction fragment length polymorphism. Using the chi-square test, we detected statistically significant differences in age (chi-square = 22.25, $P < 0.001$), gender (chi-square = 6.74, $P = 0.01$), and family history of cancer (chi-square = 4.73, $P = 0.03$) between the case and control groups. Logistic regression analysis revealed that the *XRCC1* Arg194Trp TT genotype conferred increased susceptibility to gastric cancer compared to the CC genotype [odds ratio (OR) = 2.38, 95% confidence interval (CI) = 1.28-4.49]. Moreover, individuals carrying the T allele of this variant were found to be at moderately increased risk of this disease (OR = 1.56, 95%CI = 1.16-2.09). However, the *XRCC1*

Arg399Gln and Arg280His polymorphisms were shown to have no influence on the development of gastric cancer. In conclusion, we suggest that the *XRCC1* gene Arg194Trp polymorphism is associated with gastric cancer susceptibility in the Chinese population.

Key words: *XRCC1*; Polymorphism; Gastric cancer; Chinese population

INTRODUCTION

Gastric cancer is a disease associated with high mortality, and its incidence is increasing worldwide (Ferlay et al., 2013). Poor understanding of the molecular mechanisms underlying gastric tumorigenesis has led to a lack of effective treatment (Compare et al., 2010). The etiology of this disease has not been clearly elucidated, although previous studies have shown that *Helicobacter pylori* infection can contribute to its development, in addition to that of gastric ulcers. Epidemiological research has revealed that many environmental and lifestyle factors, and family history of this condition are significantly associated with gastric cancer (van den Brandt and Goldbohm, 2006; Compare et al., 2010). Many studies have shown that genetic factors play an important role in susceptibility to gastric cancer (Lynch et al., 2005).

Previous investigations have determined that DNA repair pathways are implicated in tumorigenesis (Thompson and West, 2000; Hung et al., 2005; Wilson and Thompson, 2007). Base excision repair (BER) is key among these, and performs a vital function in the repair of small DNA lesions (Thompson and West, 2000). The gene X-ray repair complementing defective repair in Chinese hamster cells 1 (*XRCC1*) maps to chromosome 19q13.2-13.3 in humans, and is an important element of the BER pathway. *XRCC1* rectifies damage to bases and single-strand breaks in DNA induced by ionizing radiation and alkylating agents. Three common polymorphisms in the *XRCC1* protein have been identified, namely, Arg399Gln, Arg280His, and Arg194Trp. We conducted a case-control study to investigate the role of these variants in susceptibility to gastric cancer.

MATERIAL AND METHODS

Subjects

A hospital-based case-control design was employed in this investigation. A total of 214 gastric cancer patients, who were examined by gastrointestinal endoscopy and whose diagnoses were verified by pathologists, were recruited from the Department of Gastroenterology of Anhui Provincial Hospital between March 2013 and March 2015. All patients were confirmed to be free of recurrent tumors, other malignant tumors, and serious kidney and liver diseases.

During the same time period, 247 healthy subjects were recruited from among individuals having received regular health examinations at Anhui Provincial Hospital. All participants received a gastrointestinal endoscopy examination and were confirmed to be free of gastric cancer on the basis of pathological inspection. Control subjects with a history of cancer, digestive system diseases, or serious kidney or liver diseases were excluded from this study.

Exposure to potential gastric cancer risk factors was ascertained using a self-designed structured questionnaire regarding participants' gender, age, family history of cancer, alcohol consumption, and so on. Clinical data, including *H. pylori* infection and tumor-node-metastasis

stage, were collected from medical records.

A signed informed consent form was obtained from each subject prior to participation. The Ethics Committee of Anhui Provincial Hospital authorized the performance of our study, which was conducted in accordance with the Declaration of Helsinki.

Genotyping

A peripheral venous blood sample (5 mL) was obtained from each study subject and stored in a tube containing 0.5 mg/mL ethylenediaminetetraacetic acid. Genomic DNA was extracted from the collected samples using a TIANamp Blood DNA Kit (Tiangen, Beijing, China). Genotype frequencies of *XRCCI* Arg399Gln, Arg280His, and Arg194Trp polymorphisms were estimated by polymerase chain reaction (PCR)-restriction fragment length polymorphism, the primers and restriction enzymes for which are shown in Table 1. The cycling conditions used were as follows: denaturation at 94°C for 5 min, then 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s, prior to a final extension step at 72°C for 7 min. PCR products were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide, then visualized under ultraviolet light.

Table 1. Primers and restriction enzymes used to genotype *XRCCI* Arg399Gln, Arg280His and Arg194Trp polymorphisms.

Polymorphism	Primers (5'-3')	Product size(bp)	Restriction enzyme
Arg399Gln	TTGTGCTTCTCTGTGTCCA TCCTCCAGCCTTACTGATA	615	<i>MspI</i>
Arg280His	TGGGGCTGGATTGCTGGGTCTG CAGCACCCTACACACCCTGAAGG	280	<i>RsaI</i>
Arg194Trp	GCCCCGTCCCAGGTAAGC AGCCCCAAGACCCTTCA	491	<i>PvuII</i>

Statistical analysis

Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Differences in demographic variables between gastric cancer patients and control subjects were evaluated using the chi-square test. The genotype frequencies of *XRCCI* Arg399Gln, Arg280His, and Arg194Trp in the control group were tested for departure from Hardy-Weinberg equilibrium (HWE) using an exact test. Allele and genotype frequencies of each polymorphism in the two study groups were compared using the chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression analysis adjusted for potential gastric cancer risk factors. Two-sided P values < 0.05 were considered statistically significant.

RESULTS

Demographic and clinical data concerning the gastric cancer patients and control subjects are shown in Table 2. Using the chi-square test, we observed significant differences in terms of age (chi-square = 22.25, P < 0.001), gender (chi-square = 6.74, P = 0.01), and family history of cancer (chi-square = 4.73, P = 0.03) between the two groups. However, cases and controls did not differ in regard to tobacco use (chi-square = 0.91, P = 0.34). Of the 214 patients, 80 (37.38%) were at stages I-II, and 134 (62.62%) stages III-IV.

Table 2. Demographic and clinical characteristics of gastric cancer patients and control subjects.

Variable	Patients (N = 214)	%	Controls (N = 247)	%	Chi-square	P
Age, years						
<60	89	41.59	157	63.56		
≥60	125	58.41	90	36.44	22.25	<0.001
Gender						
Female	70	32.71	110	44.53		
Male	144	67.29	137	55.47	6.74	0.01
Family history of cancer						
No	196	91.59	238	96.36		
Yes	18	8.41	9	3.64	4.73	0.03
Alcohol consumption						
No	136	63.55	179	72.47		
Yes	78	36.45	68	27.53	4.21	0.04
Tobacco use						
No	141	65.89	173	70.04		
Yes	73	34.11	74	29.96	0.91	0.34
Subsite						
Non-cardia	146	68.22				
Cardia	68	31.78				
Stage						
I-II	80	37.38				
III-IV	134	62.62				

Genotype distributions of the *XRCC1* Arg399Gln, Arg280His, and Arg194Trp variants were found to be consistent with HWE ($P = 0.62$, 0.40 , and 0.06 , respectively; Table 3). Statistically significant differences in Arg194Trp genotype (chi-square = 8.88, $P = 0.01$) and allele frequencies (chi-square = 9.53, $P = 0.002$) were found between gastric cancer patients and control subjects. However, the genotype frequencies of the Arg399Gln (chi-square = 2.05, $P = 0.36$) and Arg280His (chi-square = 2.07, $P = 0.36$) variants did not significantly differ between the two groups according to the chi-square test. Using logistic regression analysis, we observed that the Arg194Trp TT genotype significantly increased susceptibility to gastric cancer compared to the CC genotype (OR = 2.38, 95%CI = 1.28-4.49). Moreover, individuals carrying the T allele of this polymorphism were at moderately increased risk of this disease (OR = 1.56, 95%CI = 1.16-2.09). However, the Arg399Gln and Arg280His *XRCC1* gene variations exhibited no influence on the development of gastric cancer in our logistic regression analysis.

DISCUSSION

As with other tumors, the occurrence of gastric cancer is closely related to defects in several DNA repair genes (Bashir et al., 2015; Ji et al., 2015). In the present study, we assessed the effect of the *XRCC1* gene Arg399Gln, Arg280His, and Arg194Trp polymorphisms on gastric cancer risk, finding that the latter may influence susceptibility to this disease.

Previous studies have reported associations between *XRCC1* sequence variations and development of several cancers, including those of the lung, colorectum, ovary, breast, skin, and prostate (Guo et al., 2015; Han et al., 2015; Hsu et al., 2015; Malisic et al., 2015; Nissar et al., 2015; Zhu et al., 2015). For example, Han et al. (2015) conducted a case-control study, reporting that the Arg399Gln polymorphism significantly elevates susceptibility to non-small cell lung cancer. Similarly, in a study of 100 colorectal cancer patients and 100 healthy controls in an Indian population, Nissar et al. (2015) suggested that individuals carrying the *XRCC1*

Table 3. Genotype and allele frequencies of *XRCCI* Arg399Gln, Arg280His, and Arg194Trp polymorphisms and their association with gastric cancer.

<i>XRCCI</i>	Patients	%	Controls	%	P for HWE	Chi-square	P	OR (95%CI) ¹	P
Arg399Gln									
GG	105	48.39	132	53.44				1.0 (Ref.)	-
GA	87	40.09	95	38.46				1.51 (0.78-1.73)	0.48
AA	25	11.52	20	8.10	0.62	2.05	0.36	1.57 (0.79-3.16)	0.17
Allele									
G	297	68.43	359	72.67				1.0 (Ref.)	-
A	137	31.57	135	27.33		2.00	0.16	1.23 (0.91-1.64)	0.16
Arg280His									
GG	135	62.21	164	66.40				1.0 (Ref.)	-
GA	66	30.41	72	29.15				1.11 (0.73-1.70)	0.60
AA	16	7.37	11	4.45	0.40	2.07	0.36	1.77 (0.74-4.36)	0.16
Allele									
G	336	77.42	400	80.97				1.0 (Ref.)	-
A	98	22.58	94	19.03		1.78	0.18	1.24 (0.89-1.73)	0.18
Arg194Trp									
CC	103	47.47	142	57.49				1.0 (Ref.)	-
CT	76	35.02	83	33.60				1.26 (0.83-1.92)	0.26
TT	38	17.51	22	8.91	0.06	8.88	0.01	2.38 (1.28-4.49)	0.003
Allele									
C	282	129.95	367	74.29				1.0 (Ref.)	-
T	152	70.05	127	25.71		9.53	0.002	1.56 (1.16-2.09)	0.002

¹Adjusted for age, gender, family history of cancer, and alcohol consumption. HWE = Hardy-Weinberg equilibrium, OR = odds ratio, CI = confidence interval, Ref. = reference.

Arg194Trp variant are at increased risk of colorectal cancer. Malisic et al. (2015) tested 50 ovarian cancer specimens and 78 samples from healthy controls, from which they concluded that the *XRCCI* Arg399Gln polymorphism may influence susceptibility to this disease. Moreover, Guo et al. (2015) established that this same variant might be correlated with increased risk of breast cancer using a meta-analysis of 13 published case-control studies. However, Hsu et al. (2015) found no significant association between *XRCCI* gene polymorphisms and skin cancer risk, although in a case-control study, Zhu et al. (2015) demonstrated that individuals carrying the Arg194Trp variation in this gene are more susceptible to prostate cancer.

Several investigators have scrutinized the association between *XRCCI* gene polymorphisms and gastric cancer risk, but their results have been inconsistent (Yan et al., 2009; Engin et al., 2011; Yuan et al., 2011; Karahalil et al., 2012; Pan et al., 2012; Wen et al., 2012). In Turkish populations, Engin et al. (2011) and Karahalil et al. (2012) found a statistically significant association between the *XRCCI* Arg399Gln gene polymorphism and gastric cancer risk in Turkish populations. Wen et al. (2012), Pan et al. (2012), and Yuan et al. (2011) revealed a significant relationship between the Arg194Trp variant and susceptibility to this disease among Chinese individuals, while Yan et al. (2009) reported that Arg399Gln and Arg194Trp sequence alterations have no effect, but the Arg280His polymorphism significantly increases gastric cancer risk. A recent meta-analysis incorporating data from 10,427 participants indicated that the Arg399Gln and Arg280His variants do not influence the occurrence of this malignancy, but the Arg194Trp polymorphism does significantly elevate risk for Asian individuals (Zhao et al., 2014). The results of our study are consistent with this meta-analysis, revealing the *XRCCI* gene Arg194Trp variant to affect susceptibility to gastric cancer. Further studies are greatly needed to confirm our findings.

Two limitations to our study should be considered. First, we were unable to divide participants into subgroups owing to the limited sample size. Second, the relatively small

study population may have reduced the statistical power of our analyses to detect differences between groups.

In conclusion, we suggest that the *XRCC1* gene Arg194Trp polymorphism may influence the development of gastric cancer in the Chinese population examined, but further studies with larger sample sizes are required to verify our results.

Conflicts of interest

The authors declare no conflict of interest.

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