Association between the c.910A>G genetic variant of the \textit{XRCC1} gene and susceptibility to esophageal cancer in the Chinese Han population

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Abstract

Esophageal cancer (EC) is a common malignancy worldwide. The X-ray repair cross-complementing 1 gene (\textit{XRCC1}) is one of the most important candidate genes for influencing susceptibility to EC. This study aimed to investigate the effect of \textit{XRCC1} genetic variants on susceptibility to EC. A total of 383 EC patients (males: 239, females: 144, mean age: 56.62) and 387 cancer-free controls (males: 251, females: 136, mean age: 58.23) were enrolled in this study. The c.910A>G genetic variant of the \textit{XRCC1} gene was determined by polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing methods. The allele and genotype frequencies indicated statistical differences between EC patients and cancer-free controls. The c.910A>G genetic variant was statistically associated with increased susceptibility to EC [GG vs AA: odds ratio (OR)=1.79, 95% confidence interval (CI)=1.12-2.86, \(P=0.014\); GG vs AG/AA: OR=1.76, 95%CI=1.13-2.75, \(P=0.013\); G vs A: OR=1.25, 95%CI=1.01-1.55, \(P=0.041\)]. The allele G and genotype GG could contribute to the increased susceptibility to EC. Our findings suggest that the c.910A>G genetic variant is associated with susceptibility to EC in the Chinese Han population, and might be used as a molecular marker for detecting susceptibility to EC.

Key words: Esophageal cancer; \textit{XRCC1} gene; Genetic variant; Molecular marker; Susceptibility

Introduction

Esophageal cancer (EC) is the eighth most common cancer globally and the sixth most common cause of cancer-related deaths worldwide, with an estimated 3.8% of all new cancer cases, and 5.4% of cancer-related deaths each year (1-5). The majority of EC cases occur in developing countries. In China, EC still remains one of the leading causes of cancer death, with approximately 250,000 cases diagnosed yearly, and it contributes to about one-half of the world's EC cases (4-9). Previous studies demonstrated that the risk of EC has been associated with various factors such as nitrosamine carcinogens, alcohol intake, cigarette smoking, malnutrition, and genetic polymorphisms (5,8,10-13). It is generally accepted that genetic susceptibility may play a key role in the pathogenesis of EC (8,14,15). Recently, several reports indicated that the X-ray repair cross-complementing 1 gene (\textit{XRCC1}) is one of the most important candidate genes for influencing EC susceptibility (4,5,7-9,12-24). The \textit{XRCC1} gene is located on chromosome 19q13.2 \(\sim\)q13.3, and has 17 exons. The \textit{XRCC1} protein plays a critical role in the repair of single-strand DNA breaks and in the DNA base excision repair pathway (4,5,7,9,16,17,19). Single genetic polymorphisms of the \textit{XRCC1} gene could impact the expression and function of the \textit{XRCC1} protein, which would influence susceptibility to EC. Evidence from published studies indicated that several single genetic polymorphisms in the \textit{XRCC1} gene, such as arginine (Arg)194 tryptophan (Trp), Arg280 histidine (His), and Arg399 glutamine (Gln), have been potentially associated with susceptibility to EC (4,5,7,8,12-19,23). However, to date, no similar studies have reported the potential association between the c.910A>G genetic variant and susceptibility to EC. Therefore, the objective of the present study was to...
investigate the distribution of the c.910A>G genetic variant and to evaluate its effect on susceptibility to EC.

**Subjects and Methods**

**Subjects**
The study consisted of 383 EC patients (males: 239, females: 144, mean age: 56.62) with a pathology-confirmed diagnosis and 387 healthy controls (males: 251, females: 136, mean age: 58.23) from The First Affiliated Hospital, Zhengzhou University, China. All subjects were of Chinese Han ethnicity. The controls were free from cancer and were frequency matched to the patients by gender and age. The general characteristics of the EC patients and cancer-free controls are summarized in Table 1, including gender, age, tobacco smoking, alcohol intake, green tea consumption, body mass index (BMI), and family history of cancer. This study was approved by the Ethics Committee of The First Affiliated Hospital, Zhengzhou University. Written informed consent forms were signed by all participants.

**Genotyping**
Genomic DNA was isolated from peripheral venous blood collected from each participant using an Axygen DNA isolation kit (Axygen, USA). One pair of specific polymerase chain reaction (PCR) primers (F: 5'−GC CTGGACTGCTGGGTCTGAG-3'; R: 5'−TCAGCACCA CTACCACCCCTG-3') was designed by the Primer Premier 5.0 software (Premier Biosoft International, USA). PCR was performed in a total volume of 20 μL containing 50 ng template DNA, 1× buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 0.25 μM primers, 2.0 mM MgCl$_{2}$, 0.25 mM dNTPs, and 0.5 U Taq DNA polymerase (Promega, USA). The PCR protocol was performed at 94°C for 5 min, followed by 32 cycles at 94°C for 30 s, 64.2°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 8 min. The genotyping of the c.910A>G genetic variant of the XRCC1 gene was analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP). Following the supplier’s instructions, aliquots of 5-μL amplified PCR products were digested with 2 U HhaI restriction enzyme (MBI Fermentas, Germany) at 37°C for 10 h. The digested products were separated by 2.5% agarose gel electrophoresis containing ethidium bromide and visualized under ultraviolet light. To confirm the accuracy of the PCR-RFLP genotyping results, 10% random samples were re-analyzed by DNA sequencing methods (ABI3730xl DNA Analyzer, Applied Biosystems, USA).

Table 1. General characteristics of patients with esophageal cancer and cancer-free controls.

| Characteristics                  | Patients | Controls | χ²   | P   |
|----------------------------------|----------|----------|------|-----|
| Number                           | 383 (49.7%) | 387 (50.3%) | 0.5017 | 0.4788 |
| Gender                           |          |          |      |     |
| Male                             | 239 (62.4%) | 251 (64.9%) |      |     |
| Female                           | 144 (37.6%) | 136 (35.1%) |      |     |
| Age (years)                      |          |          | 1.6647 | 0.1970 |
| Means ± SD                       | 56.62 ± 10.89 | 58.23 ± 11.36 |      |     |
| <60                              | 172 (44.9%) | 156 (40.3%) |      |     |
| >60                              | 211 (55.1%) | 231 (59.7%) |      |     |
| Tobacco smoking                  |          |          | 3.0090 | 0.0828 |
| Yes                              | 209 (54.6%) | 187 (48.3%) |      |     |
| No                               | 174 (45.4%) | 200 (51.7%) |      |     |
| Alcohol intake                   |          |          | 0.4000 | 0.5271 |
| Yes                              | 152 (39.7%) | 145 (37.5%) |      |     |
| No                               | 231 (60.3%) | 242 (62.5%) |      |     |
| Green tea consumption            |          |          | 0.5481 | 0.4591 |
| Yes                              | 166 (43.3%) | 178 (46%) |      |     |
| No                               | 217 (56.7%) | 209 (54%) |      |     |
| Body mass index                  |          |          | 0.4599 | 0.4977 |
| <23                              | 158 (41.3%) | 169 (43.7%) |      |     |
| >23                              | 225 (58.7%) | 218 (56.3%) |      |     |
| Family history of cancer         |          |          |      |     |
| Yes                              | 83 (21.7%) | – |      |     |
| No                               | 300 (78.3%) | – |      |     |

Data are reported as number with percent in parentheses. There were no significant differences between patients and cancer-free controls (P>0.05, χ² test).
Statistical analysis
All statistical analyses were carried out using the SPSS software (Windows version 15.0; SPSS Inc., USA). The chi-square test was used to evaluate the Hardy-Weinberg equilibrium (HWE) in genotypic distributions and to compare differences in general characteristics between EC patients and cancer-free controls. For the association between allele/genotype and EC risk, we estimated the odds ratios (ORs) and their 95% confidence intervals (CIs) by unconditional logistic regression. P values of <0.05 were considered to be statistically significant.

Results
Subjects and general characteristics
There were no significant differences between EC patients and cancer-free controls in gender, age, tobacco smoking, alcohol intake, green tea consumption, and BMI (Table 1; P >0.05).

Identification of the XRCC1 genetic variant
Genotyping of the c.910A>G genetic variant of the XRCC1 gene was identified in this study using PCR-RFLP and DNA sequencing methods. Our sequence analyses suggested that the c.910A>G genetic variant is caused by an A to G mutation, and this is a nonsynonymous mutation corresponding to a threonine (Thr) to alanine (Ala) amino acid replacement in exon 9 of the XRCC1 gene (p.Thr304Ala, reference sequences: GenBank IDs: NC_000019.9, NM_006297.2, and NP_006288.2). The HhaI restriction enzyme was selected to digest PCR-amplified products of the c.910A>G genetic variant. Three possible genotypes were defined by three distinct banding patterns: AA (243 bp), AG (243, 171, and 72 bp), and GG (171 and 72 bp).

Allelic and genotypic frequencies
Table 2 reports the allelic and genotypic frequencies in the study subjects. Both allele A and genotype AA had maximum frequencies in EC patients and cancer-free controls. The allele frequencies in EC patients (A, 65.14%; G, 34.86%) were significantly different from those in cancer-free controls (A, 70.03%; G, 29.97%; \( \chi^2 = 4.1896, P = 0.0407 \), Table 2). The genotype frequencies in EC patients (AA, 45.17%; AG, 39.95%; GG, 14.88%) were not consistent with cancer-free controls (AA, 49.10%; AG, 41.86%; GG, 9.04%), the differences being statistically significant (\( \chi^2 = 6.2935, P = 0.0430 \), Table 2). The \( \chi^2 \) test for genotype distributions in the study populations indicated that this genetic variant did not significantly deviate from the HWE (all P values >0.05).

Association between the XRCC1 genetic variant and risk of EC
The potential association of the XRCC1 c.910A>G genetic variant with EC risk is shown in Table 3. Significantly increased risks of EC were detected in homozygote comparisons, recessive models, and contrasting alleles.

Discussion
EC is a common and polygenic malignant cancer caused by complex interactions between genetic and environmental factors. It is a global health problem. A large number of studies have been conducted on genetic variants of candidate genes that play key roles in the pathogenesis of EC (8,14,15). In the present study, the influence of the c.910A>G genetic variant of the XRCC1 gene on EC risk was evaluated by association analysis in 383 EC patients and 387 cancer-free Chinese subjects with Han ethnicity. Our data indicated that the distributions of allele and genotype frequencies in EC patients were significantly different from those of cancer-free controls (all P values <0.05; Table 2). The genotype GG was statistically associated with increased EC risk compared with genotype AA and AG/AA carriers (all P values <0.05; Table 3). Compared with allele A, allele G might be an increased genetic risk factor for EC. Results from this study provided more evidence of the role of the XRCC1 gene in the development of EC and suggested that the c.910A>G genetic variant of the XRCC1 gene was statistically associated with EC risk in the Chinese Han population; therefore, this variant could be used as a...
Table 3. Association between esophageal cancer risk and the c.910A->G genetic variant of the XRCC1 gene.

| Comparisons                        | Test of association |
|------------------------------------|--------------------|
|                                    | OR (95%CI) | $\chi^2$ | P    |
| Homozygote comparison (GG vs AA)   | 1.79 (1.12-2.86) | 5.99     | 0.014 |
| Heterozygote comparison (AG vs AA) | 1.04 (0.77-1.40) | 0.06     | 0.813 |
| Dominant model (GG/AG vs AA)       | 1.17 (0.88-1.55) | 1.19     | 0.276 |
| Recessive model (GG vs AG/AA)      | 1.76 (1.13-2.75) | 6.23     | 0.013 |
| Allele contrast (G vs A)           | 1.25 (1.01-1.55) | 4.19     | 0.041 |

OR: odds ratio; CI: confidence interval. The $\chi^2$ test was used for statistical analyses.

molecular marker for evaluating susceptibility to EC. Our findings are consistent with several similar studies that demonstrated the potential association between other XRCC1 genetic variants and the risk of EC (for example, Arg194Trp, Arg280His, and Arg399Gln). Cai et al. (16) indicated that the Arg194Trp genetic polymorphism may be associated with an increased risk of developing esophageal squamous cell carcinoma (ESCC). Xing et al. (7) found that individuals with the Trp/Trp genotype at the XRCC1 Arg194Trp site had a 2-fold increased risk of ESCC compared with the Arg/Arg genotype (adjusted OR = 1.98, 95% CI = 1.26-3.12). Zhai et al. (17) showed that, regarding the XRCC1 codon 280 His allele, there was no significant difference between ESCC patients and normal controls (P > 0.05). Sobti et al. (18) suggested that the XRCC1 codon 399 Gln/Gln genotype was significantly associated with reduced risk of ESCC (OR = 0.31, 95% CI = 0.12-0.78, P < 0.01). Yu et al. (8) reported that the XRCC1 codon 399 Gln/Gln genotype was associated with an increased risk of ESCC (OR = 5.15, 95% CI = 2.42-0.93), and the risk for smokers increased 4.2-fold compared with non-smokers in the 399 Gln/Gln genotype (OR = 4.20, 95% CI = 2.37-7.44). Yin et al. (15) suggested that the XRCC1 Arg399Gln polymorphism may be a potential biomarker for EC susceptibility in Chinese populations, particularly for squamous cell carcinoma. Cai et al. (16) demonstrated that XRCC1 Gln variant alleles were associated with an increased risk of ESCC with adjusted ORs of 1.67 (95% CI = 1.08-2.59). Yu et al. (4) found that both lifestyle-related factors - such as drinking river water, consuming long-term stored rice, and alcohol intake - and the XRCC1 Arg399Gln polymorphism were possible risk factors for ESCC among Chinese people. These observations demonstrated that genetic variants of the XRCC1 gene may contribute to genetic effects on the development of EC.

In conclusion, the current study indicates that the c.910A->G genetic variant of the XRCC1 gene may influence the risk of EC. It would be necessary to confirm our findings in large functional studies with different ethnic populations and to elucidate the underlying molecular mechanisms for the etiology of EC.

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