Research Article

Interaction of XRCC1 Arg399Gln Polymorphism and Alcohol Consumption Influences Susceptibility of Esophageal Cancer

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Background. To explore the correlation between the Arg399Gln polymorphism and susceptibility to esophageal cancer in Korean and Han Chinese individuals in Harbin, China, and its potential interaction with alcohol consumption. Methods. This prospective study included 203 patients with esophageal squamous cell carcinoma; 88 were of Korean descent and 115 were of Han Chinese descent. A group of healthy controls included 105 participants of Korean descent and 105 of Han Chinese descent. Genotyping of the Arg399Gln locus of XRCC1 was performed by PCR-RFLP. Results. The allelic and genotypic frequencies were not significantly different between individuals with esophageal cancer and controls or between individuals of Korean and Han Chinese descent (P > 0.05). However, when individuals with the wild-type Arg/Arg genotype also consumed alcohol, the risk of esophageal cancer was lower (OR = 3.539; 95% CI = 2.039–6.142; P < 0.05). Conclusions. The XRCC1 Arg399Gln polymorphism does not appear to be associated with esophageal cancer in individuals of Korean or Han Chinese descent in Harbin, China. However, alcohol consumption may decrease the risk of esophageal cancer in persons with the wild-type genotype.

1. Introduction

Many cancers result from damage to DNA that eventually affects DNA stability and leads to the malignant transformation of cells. DNA damage must be repaired by one of four cellular processes, base excision repair, nucleotide excision repair, mismatch repair, or double-strand break repair, and alterations in the repair process can allow DNA damage to accumulate [1]. The DNA damage repair gene XRCCI (X-ray repair crosscomplementing gene 1) functions in base excision repair and the repair of single-strand breaks that are caused by ionizing radiation and oxidative damage [2]. Importantly, polymorphisms in XRCCI are correlated with susceptibility to various tumors [3–6].

Esophageal cancer (EC), a common malignancy of the digestive tract, has a complex etiology and is currently believed to result from combined genetic and environmental factors [7]. Several genes, including XRCCI, are associated with EC risk [8]. Environmental factors including smoking history, alcohol consumption, and nutrition have also been associated with EC risk [9]. At least one polymorphism in XRCCI, Arg194Trp, has been associated with the occurrence of EC; in contrast, the Arg280His variant appears not to be associated with the occurrence of EC, and conflicting reports have made the contribution of the Arg399Gln variant to the disease unclear [10–13]. Further, potential interactions between XRCCI variants and environmental factors have not been resolved. Indeed, in a population with a family history of EC, affected individuals with exposure to the same environmental factors account for just a small proportion [14, 15]. This suggests that individual genetic susceptibility to environmental exposures is associated with the occurrence of EC; thus, interaction(s) between genetic susceptibility and environmental factors likely contribute to EC etiology.

To investigate the potential interaction(s) between genetic variation and an environmental factor, alcohol consumption, Korean and Han Chinese individuals with esophageal cancer were selected from the Harbin area of China, which is inhabited by more Korean people. The distribution of the Arg399Gln polymorphism of XRCCI was
identified, and genotypes were studied in relation to both esophageal cancer and alcohol consumption.

### 2. Participants and Methods

#### 2.1. Participants
This prospective study included 203 patients with esophageal cancer (case group), who visited the Harbin Medical University Cancer Hospital between July 2013 and June 2014. Of these, 124 were males, and 79 were females; 115 were Han Chinese and 88 were Korean. Their mean age was 60.7 ± 8.1 years. All patients underwent surgery or gastroscopy and were pathologically confirmed to have esophageal squamous cell carcinoma. Additionally, other possible bodily tumors were ruled out, and patients did not undergo radiotherapy or chemotherapy. For the control group, 210 patients with nondigestive disorders who were hospitalized during the same period were randomly selected. Of them, 113 were males and 97 were females; 115 were Han Chinese and 105 were Korean. The mean age was 58.6 ± 6.5 years. The control and EC groups were not significantly different in age or sex distribution ($P > 0.05$). All participants provided informed consent. This study was approved by the Ethics Committee of the Harbin Medical University Cancer Hospital.

Peripheral blood (5 mL) was collected from each subject into EDTA-coated tubes and stored at −80°C. Alcohol consumption history was collected from both groups using questionnaires. Participants were considered as alcohol consumers if they consumed an alcoholic beverage once per week and of at least 40 mL of alcohol.

#### 2.2. Genomic DNA Extraction
DNA extraction from whole blood was performed according to the instructions in the AxyPrep blood genomic DNA kit (Axygen Biosciences, CA, USA), as follows: 5 mL of peripheral blood was anticoagulated with sodium citrate to isolate and lyse white blood cells. Sodium precipitation was used to extract DNA, and extracted DNA was stored at −20°C.

#### 2.3. Genotyping
Genotypes for *XRCC1* were determined by PCR-restriction fragment length polymorphism. Primer sequences for *XRCC1* amplification were designed and synthesized by Sangon Biotech (Shanghai, China). The upstream primer for Arg399Gln was 5′-TTGTGCTTTCTCTTGTTCCA-3′, the downstream primer was 5′-TCCTTCCAGCCTTTTCTGATA-3′, and the anticipated product was 615 bp. PCR amplification was performed in a PCR Thermal Cycler Dice (TaKaRa Biotech Co. Ltd, Code TP600, Dalian, China) using the following conditions: pre-denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s, and extension at 72°C for 45 s; and extension at 72°C for 7 min. PCR products were subjected to restriction digestion with *Msp I* (TaKaRa Biotech Co. Ltd, Dalian, China) overnight at 37°C in a water bath. Five internal positive controls were prepared at the same time. Digested products were separated by agarose gel electrophoresis (2%) and visualized by a gel imaging system.

#### 2.4. Statistical Treatment
SPSS 17.0 was used to analyze the data. Measurement data are expressed as mean ± standard deviation. A chi-square test was used to compare the allelic and genotypic frequencies in the case group and control group, and the genotype distributions in both groups were compared using a chi-square contingency table. Odds ratios (OR) and 95% confidence intervals (CI) were used to study the correlation of various genotypes and alcohol consumption with the risk of esophageal cancer.

### 3. Results

#### 3.1. XRCC1 Arg399Gln Genotype Distributions in Esophageal Cancer Cases and Controls of Korean and Han Chinese Descent
Hardy-Weinberg equilibrium was used to test the genotype distributions of the *XRCC1* Arg399Gln locus in both Korean and Han Chinese populations. The three possible genotypes were Arg/Arg, Arg/Gln, and Gln/Gln (Figure 1). For each group, the distributions met Hardy-Weinberg equilibrium.

In comparing the EC group with the control group, the allelic and genotypic frequencies at Arg399Gln were not significantly different ($P > 0.05$; Table 1). The gene mutation was considered an exposure and the wild-type genotype was the control; there were no marked differences in the occurrence of EC in subjects with various genotypes. Further, the allelic and genotypic frequencies were compared in individuals with EC of Korean and Han Chinese descent, and there were no significant differences between the groups ($P > 0.05$; Table 2). Additionally, the allelic and genotypic
Table 1: Analysis of correlation between the XRCC1 Arg399Gln polymorphism and susceptibility to esophageal cancer (n (%)).

| Groups        | n  | Arg/Arg | Arg/Gln | Gln/Gln | A   | G   |
|---------------|----|---------|---------|---------|-----|-----|
| Case group    | 203| 102 (50.2) | 78 (38.4) | 23 (11.3) | 282 (69.5) | 124 (30.5) |
| Control group | 210| 125 (59.5) | 63 (30.0)  | 22 (10.5)  | 313 (74.5)  | 107 (25.5) |
| \(\chi^2\)    |    | 3.831  |         |         |     |     |
| \(P\)         |    | 0.147  |         |         |     |     |

Table 2: Analysis of correlation between the XRCC1 Arg399Gln polymorphism and susceptibility to esophageal cancer by nationality (n (%)).

| EC population | n  | Arg/Arg | Arg/Gln | Gln/Gln | A   | G   |
|---------------|----|---------|---------|---------|-----|-----|
| Han Chinese   | 115| 55 (47.8) | 48 (41.7) | 12 (10.4) | 148 (67.3) | 72 (32.7) |
| Korean        | 88 | 47 (53.4) | 30 (34.1) | 11 (12.5) | 124 (70.5) | 52 (29.5) |
| Total         | 203| 102 | 78 | 23 | 282 | 124 |
| \(\chi^2\)    |    | 1.256  |         |         |     |     |
| \(P\)         |    | 0.534  |         |         |     |     |

Table 3: The interaction between alcohol consumption history and XRCC1 Arg399Gln polymorphism (n (%)).

| Alcohol consumption history | Genotype | Control group | Case group | OR         | 95% CI      | \(\chi^2\) | \(P\) |
|----------------------------|----------|---------------|------------|------------|-------------|------------|------|
| Yes                        | Arg/Arg  | 88 (68.2) | 41 (31.8) | 3.539      | 2.039–6.142 | 20.886    | 0.000|
|                            | Arg/Gln  | 39 (48.1) | 42 (51.9) | 0.653      | 0.359–1.187 | 1.961     | 0.161|
|                            | Gln/Gln  | 14 (46.7) | 16 (53.3) | 0.693      | 0.304–1.582 | 0.761     | 0.383|
|                            | AG + GG  | 53 (56.1) | 58 (43.9) | 0.664      | 0.382–1.154 | 2.120     | 0.145|
| No                         | Arg/Arg  | 37 (37.8) | 61 (62.2) | Reference  |             |           |      |
|                            | Arg/Gln  | 24 (40.0) | 36 (60.0) | 0.910      | 0.471–1.758 | 0.079     | 0.778|
|                            | Gln/Gln  | 8 (53.3)  | 7 (46.7)  | 0.531      | 0.178–1.584 | 1.317     | 0.251|
|                            | AG + GG  | 30 (47.6) | 33 (52.4) | 0.667      | 0.351–1.267 | 1.536     | 0.215|

frequencies by nationality were compared between EC cases and controls; no significant difference was detected.

3.2. Interaction between Alcohol Consumption and XRCC1 Arg399Gln Polymorphism on Risk of EC. Within the EC group, participants were stratified by genotype and alcohol consumption history (Table 3). The reference group comprised individuals with the wild-type Arg/Arg genotype who did not consume alcohol. Of those who consumed alcohol, the odds ratios (95% CI) for the Arg/Arg and Arg/Gln genotypes were 3.539 (2.039–6.142; \(P < 0.05\)) and 0.653 (0.359–1.187; \(P > 0.05\)), respectively.

4. Discussion

Previous research has produced conflicting results regarding the contribution of the XRCC1 Arg399Gln polymorphism to the risk of esophageal cancer [10–13]. One study demonstrated an increased risk of EC in individuals with the Gln/Gln genotype, and the Arg399Gln polymorphism was correlated with susceptibility to EC [10]. In contrast, our study identified no differences in the genotypic distributions between individuals with EC and healthy controls. This finding supports those of other studies [11, 13]. The difference in results between this/similar studies [11, 13] and conflicting studies [10, 12] may result from variations in sample size or geographic distributions. Additionally, confounding factors like lifestyle or environmental influences were not investigated in all prior studies. No increased likelihood of EC was identified for any genotype, which indicates that the XRCC1 Arg399Gln polymorphism may not be correlated with EC in Harbin city (Heilongjiang Province, China). In addition, the genotypic distributions did not differ among Korean and Han Chinese nationalities.

Because previous studies did not assess interactions between environmental factors and XRCC1 genotype on EC risk, alcohol consumption history was investigated as a potential interacting factor here. Interestingly, compared with individuals with EC who had a wild-type genotype and did not consume alcohol, those who consumed alcohol were ∼3.5 times less likely to develop EC if they had the wild-type Arg/Arg genotype.

Thus, the findings of this study corroborate previous results indicating that the XRCC1 Arg399Gln polymorphism does not contribute to EC risk. However, alcohol consumption may be an important interacting factor that decreases EC risk in individuals with the wild-type genotype.
Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] C. Latif, S.-H. Harvey, and M.-J. O'Connell, "Ensuring the stability of the genome: DNA damage checkpoints," TheScientificWorldJournal, vol. 1, pp. 684–702, 2001.

[2] R. Brem and J. Hall, "XRCC1 is required for DNA single-strand break repair in human cells," Nucleic Acids Research, vol. 33, no. 8, pp. 2512–2520, 2005.

[3] G. Liu, W. Zhou, B. Y. Yeap et al., "XRCC1 and XPD polymorphisms and esophageal adenocarcinoma risk," Carcinogenesis, vol. 28, no. 6, pp. 1254–1258, 2007.

[4] U. Warnecke-Eberz, D. Vallböhmer, H. Alakus et al., "ERCC1 and XRCC1 gene polymorphisms predict response to neoadjuvant radiochemotherapy in esophageal cancer," Journal of Gastrointestinal Surgery, vol. 13, no. 8, pp. 1411–1421, 2009.

[5] H. Yu, C. Fu, J. Wang, H. Xue, and B. Xu, "Interaction between XRCC1 polymorphisms and intake of long-term stored rice in the risk of esophageal squamous cell carcinoma: a case-control study," Biomedical and Environmental Sciences, vol. 24, no. 3, pp. 268–274, 2011.

[6] X.-Q. Chen, F. Wang, Y.-L. Zheng, Q. X. Fan, D. L. Yue, and Z. J. Ma, "Association between the c.910A>G genetic variant of the XRCC1 gene and susceptibility to esophageal cancer in the Chinese Han population," Brazilian Journal of Medical and Biological Research, vol. 46, no. 12, pp. 1028–1032, 2013.

[7] K. K. Keditsu, S. Jiwnani, G. Karimundackal, and C. S. Pramesh, "Multimodality management of esophageal cancer," Indian Journal of Surgical Oncology, vol. 4, no. 2, pp. 96–104, 2013.

[8] C. E. Denlinger and R. K. Thompson, "Molecular basis of esophageal cancer development and progression," Surgical Clinics of North America, vol. 92, no. 5, pp. 1089–1103, 2012.

[9] M.-A. Henry, M.-M. Lerco, P.-W. Ribeiro, and M.-A. Rodrigues, "Epidemiological features of esophageal cancer: Squamous cell carcinoma versus adenocarcinoma," Acta Cirurgica Brasileira, vol. 29, no. 6, pp. 389–393, 2014.

[10] B. Hao, H. Wang, K. Zhou et al., "Identification of genetic variants in base excision repair pathway and their associations with risk of esophageal squamous cell carcinoma," Cancer Research, vol. 64, no. 12, pp. 4378–4384, 2004.

[11] J. Huang, J. Zhang, Y. Zhao et al., "The Arg194Trp polymorphism in the XRCC1 gene and cancer risk in Chinese Mainland population: a meta-analysis," Molecular Biology Reports, vol. 38, no. 7, pp. 4565–4573, 2011.

[12] Z.-Y. Zhang, Y. Xuan, X.-Y. Jin, X. Tian, and R. Wu, "Meta-analysis demonstrates association of XRCC1 genetic polymorphism Arg399Gln with esophageal cancer risk in the Chinese population," Genetis and Molecular Research, vol. 12, no. 3, pp. 2567–2577, 2013.

[13] S. Li, Y. Deng, J.-P. You et al., "XRCC1 Arg399Gln, Arg194Trp, and Arg280His polymorphisms in esophageal cancer risk: a meta-analysis," Digestive Diseases and Sciences, vol. 58, no. 7, pp. 1880–1890, 2013.

[14] Y. Vinogradova, C. Coupland, and J. Hippisley-Cox, "Exposure to bisphosphonates and risk of gastrointestinal cancers: series of nested case-control studies with QResearch and CPRD data," British Medical Journal, vol. 346, no. 7894, article f114, 2013.

[15] Y. Yokokawa, S. Ohta, J. Hou et al., "Ecological study on the risks of esophageal cancer in Ci-Xian, China: the importance of nutritional status and the use of well water," International Journal of Cancer, vol. 83, no. 5, pp. 620–624, 1999.