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Original Article

Association of DNA repair relevant gene polymorphisms with the risk of gastric cancer in a Han Chinese population

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Abstract: Objective: To investigate the associations of five non-synonymous variants of four DNA repair-relevant genes with gastric cancer (GC) in a large Han Chinese population. Methods: A hospital-based case-control study, comprised of 622 patients diagnosed with gastric cancer and 622 age- and gender-matched controls, was conducted. Blood samples were collected from patients and healthy control subjects to analyze polymorphisms of XRCC1 rs1799782, rs25487, XRCC3 rs861539, hOGG1 rs1052133, and NQO1 rs1800566. Risk estimates were expressed as odds ratios (OR) and 95% confidence intervals (95% CI). Results: All five examined polymorphisms met Hardy-Weinberg equilibrium. Overall, significant differences were found in the genotype/allele distributions of rs1799782 in XRCC1, and rs1800566 in NQO1 ($P < 0.05$ for both), whereas no significant difference was observed among the other three variants between patients and controls ($P > 0.05$). The risk of gastric cancer associated with the rs1800566-T mutant allele was higher by 71% (95% CI [confidence interval]: 1.48-1.99; $P < 0.001$) under the additive model, and by 86% (95% CI: 1.48-2.33; $P < 0.001$) under the dominant model. The mutant-T allele of variant rs1799782 conferred increased GC risks of 27% (95% CI: 1.06-1.52; $P = 0.009$) and 42% (95% CI: 1.13-1.77; $P = 0.002$) under the additive and dominant models, respectively. Conclusions: Results suggest that the XRCC1 rs1799782 and NQO1 rs1800566 polymorphisms confer high susceptibility to GC in the Chinese population.

Keywords: Gastric cancer, DNA repair, association, polymorphism

Introduction

Gastric cancer (GC) is one of the most common forms of cancer. As of 2014, it was the fifth most common cancer and the third most common cause of cancer-related deaths worldwide [1]. Gastric carcinogenesis is a complex, multifactorial, multi-step, and complicated process caused by multiple environmental factors, including a high salt diet, consumption of tobacco and alcohol, and *Helicobacter pylori* infection [2]. However, different individuals exposed to the same environmental risk factors have different susceptibilities to GC [3], which suggests that the genetic underpinnings of GC may play an important role in its occurrence [4].

Oxidative stress plays a causal role in the molecular processes leading to GC [5]. Oxidative stress causes cellular macromolecule damage, which leads to DNA and protein modification, as well as lipid peroxidation [6]. GC patients are characterized by increased protein oxidation, DNA damage, and decreased lipid peroxidation [7]. Moreover, detectable oxidative DNA damage is recognized as a potential early biomarker of *H. pylori*-associated gastric carcinogenesis [5].

Here we hypothesized that genetic defects in proteins involved in DNA repair mechanisms may confer GC risk. To test this hypothesis, we assessed the associations of five non-synonymous variants of the following four DNA repair-relevant genes with GC in a large Han Chinese population: X-ray cross-complementing group 1 (XRCC1: Arg194Trp or rs1799782 and Arg-399Gln or rs25487), X-ray cross-complementing group 3 (XRCC3: Thr241Met or rs861539), oxoguanine glycosylase 1 (hOGG1: Ser326Cys or rs1052133), and NQO1 (Pro187Ser or rs1800566).

Table 1. Baseline characteristics of study population

Characteristics	GC patients (n = 622)	Controls (n = 622)	*P*
Age (years)	63.7±11.5	63.3±11.9	0.128
Gender			
Female	259 (41.6%)	265 (42.6%)	0.774
Male	363 (58.4%)	357 (57.4%)	

Materials and methods

Study design

This hospital-based case-control study comprised of 1244 subjects recruited from Ninth People's Hospital, Shanghai Jiao Tong University and School of Medicine from May 2011 to December 2015. The study population included 622 unrelated cases with histopathologically confirmed GC, and 622 age- and gender-matched cancer-free controls. All subjects were local residents of Han descent. This study was approved by the Ethics Committee of Shanghai Jiaotong University School of Medicine and was conducted according to the Declaration of Helsinki Principles. All subjects provided written informed consent.

Genotyping

Genomic DNA was prepared from whole blood using a standard phenol-chloroform method and stored at -40°C before mass genotyping. The genotypes of five examined variants were determined by the polymerase chain reaction-ligase detection reaction (PCR-LDR) method [8, 9].

The PCR reactions were conducted in an EDC-810 Amplifier (Dongsheng Innovation Biotech Co., Ltd., Beijing, China). The cycling parameters were as follows: 94°C for 2 min; 35 cycles of 94°C for 20 s, 60°C for 20 s, and 72°C for 20 s; and a final extension step at 72°C for 3 min.

We synthesized two specific probes and one common probe to discriminate specific bases of each variant. The common probe was labeled with 6-carboxy-fluorescein at the 3' end and phosphorylated at the 5' end. The multiplex ligation reaction was conducted in a reaction volume of 10 μl that contained 2 μl of PCR product, 1 μl of 10× Taq DNA ligase buffer, 1 μM of each discriminating probe, and 5 U of Taq DNA ligase. The ligation parameters were 30 cycles of 94°C for 30 s and 56°C for 3 min. Afterward, 1 μl of LDR reaction product was mixed with 1 μl of ROX passive reference and 1 μl of loading buffer, before being denatured at 95°C for 3 min and chilled rapidly in ice water. The fluorescent products of LDR were differentiated using an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA).

The accuracy of the PCR-LDR method was tested in 96 randomly selected DNA samples, which were genotyped again for quality control and found to have complete concordance.

Statistical analyses

Statistical analyses were performed with SPSS for Windows (version 17, SPSS, Inc., Chicago, IL). Differences between means of two continuous variables were evaluated by Student's t test. The chi-square test (χ^2 test) was used to compare gender distribution, examine the association between genotypes and alleles in relation to cases and controls, and test for deviation of genotype distribution from the Hardy-Weinberg equilibrium (HWE). The odds ratios (OR) and their 95% confidence intervals (CI) were calculated to estimate the strength of the association between polymorphism genotypes and alleles in patients and controls. $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics

We studied 622 GC cases (363 male and 259 female) with a mean age of 63.7±11.5 years, and 622 healthy controls (357 male and 265 female) with a mean age of 63.3±11.9 years. The demographics in the study population are summarized in **Table 1**. No significant differences in age ($P = 0.128$) and gender ($P = 0.7740$) were found between GC patients and controls.

Association between genotypes and allele frequencies of SNPs and GC risk

The genotype distributions and allele frequencies of five examined variants from GC patients and healthy controls are compared in **Table 2**. The genotype frequency of the five variants, in

Table 2. Genotype distributions and allele frequencies of five examined polymorphisms between patients and controls, and their prediction for GC

Gene polymorphism		GC patients (n = 622)	Controls (n = 622)	P_{x2}	OR	95% CI	P^*
XRCC1: rs1799782	CC	269	323		1.27	1.06-1.52	0.009
Genotype (n):	CT	304	254	0.008328	1.42	1.13-1.77	0.002
	TT	49	45		1.10	0.72-1.67	0.668
Allele (%)	T	32.32	27.65	0.01115			
XRCC1: rs25487	GG	358	329		0.87	0.73-1.03	0.104
Genotype (n):	GA	216	237	0.245	0.83	0.66-1.04	0.098
	AA	48	56		0.85	0.57-1.26	0.413
Allele (%)	A	25.08	28.05	0.09307			
XRCC3: rs861539	CC	545	562		1.26	0.92-1.72	0.153
Genotype (n):	CT	70	54	0.3008	1.32	0.93-1.89	0.125
	TT	7	6		1.17	0.93-1.89	0.781
Allele (%)	T	6.75	5.31	0.1295			
HOGG1: rs1052133	GG	220	214		0.94	0.8-1.1	0.467
Genotype (n):	GC	300	294	0.6669	0.96	0.76-1.21	0.721
	CC	102	114		0.87	0.65-1.17	0.369
Allele (%)	C	40.51	41.96	0.4635			
NQO1: rs1800566	CC	215	308		1.71	1.48-1.99	< 0.001
Genotype (n):	CT	213	226	< 0.0001	1.86	1.48-2.33	< 0.001
	TT	194	88		2.75	2.07-3.65	< 0.001
Allele (%)	T	48.31	32.32	< 0.0001			

Abbreviations: OR, odds ratio; 95% CI: 95% confidence interval; *P* values were calculated under the additive (the upper), dominant (the middle), and recessive (the lower) models of inheritance. *P** , *P* values for 95% CI.

both patients and controls, conformed to expectations under the HWE (P < 0.05).

Significant difference was observed between the patients and controls in genotype (P = 0.008) and allele (P = 0.011) distributions of variant rs1799782 of the XRCC1 gene; its mutant T allele was overrepresented in patients relative to controls (32.32% versus 27.65%). Analysis of the rs1800566 polymorphism for NQO1 gene in the GC group showed that 215 (34.6%) were homozygous for the C/C genotype, 194 (31.2%) were variant homozygous for the T/T genotype, and 213 (34.2%) were heterozygous for the C/T genotype. Significant difference existed between the case and control groups in the genotype (P < 0.001) and allele (P < 0.001) distributions. No significance was observed for the other three variants at a significance level of 5%.

Risk prediction of five examined variants of GC under three genetic models is provided in **Table 2**. Statistical analysis revealed significant association between rs1800566 polymorphism and risk of GC under the additive (OR: 1.71 95% CI: 1.48-1.99; P < 0.001), dominant (OR: 1.86 95% CI: 1.48-2.33; P < 0.001), and recessive models (OR: 2.75 95% CI: 2.07-3.65; P < 0.001). The mutant genotype or allele of variant rs1799782 conferred 27% (95% CI: 1.06-1.52; P = 0.009) increased risk of GC under the additive model, and 42% (95% CI: 1.13-1.77; P = 0.002) under the dominant model. hOGG1 Ser326Cys, XRCC3 Thr241Met, and XRCC1 Arg399Gln polymorphisms showed no significant association with GC occurrence.

Discussion

We investigated the association between five non-synonymous variants of four DNA repair-relevant genes and GC in a large Han Chinese population. We found that the XRCC1 rs1799782 and NQO1 rs1800566 polymorphisms reflected pronounced susceptibility to GC in the Chinese population. To the best of our knowledge, this is the first study to report the association of multiple DNA repair-relevant genes and polymorphisms with gastric cancer risk in a large Chinese population.

The selection of genes related to DNA repair in this association study has a strong biological basis. Increasing evidence suggests that oxidative DNA damage is involved in the pathogenesis of GC [5, 7, 10-12]. *H. pylori*, which is the leading risk factor associated with gastric carcinogenesis, is known to cause enhancement of reactive oxygen species (ROS) levels from both neutrophil infiltration and *de novo* synthesis in epithelial cells both *in vivo* and *in vitro* [13, 14]. The increase in ROS is directly linked to oxidative DNA damage, deamination, and alkylation. Without proper control of oxidative stress and DNA damage repair, neoplastic transformation leading to cancer can occur. *hOGG1* is responsible for repairing 8-hydroxy-deoxyguanosine residue, the major form of oxidative DNA damage induced by ROS [15], which makes it a logical candidate gene for GC. Consistent with this idea, mice deficient in NQO1 has been shown to exhibit significantly higher susceptibility to cancer [16, 17].

XRCC is the family of DNA repair genes responsible for repair of DNA base damage and single-strand breaks. These genes are polymorphic, with several non-synonymous polymorphisms, such as Arg194Trp and Arg399Gln in the XRCC1 gene, and Thr241Met in the XRCC3 gene. Numerous studies have attempted to associate these polymorphisms with GC occurrence but have produced conflicting results, perhaps because of population differences [18-20]. Their results were not replicable in the Chinese population, either. Shen et al. [21] conducted a population-based case-control study in central Jiangsu Province, and found that the rs1799782 CC and rs25487 GA/AA genotypes may contribute to increased risk of GC occurrence, particularly of the cardia type. Gong et al. [22] reported that rs25487, but not rs1799782, may play a major role in enhancing susceptibility to GC. In 2009, Yan and his colleagues [23] failed to find association between gastric cardiac adenocarcinoma with rs25487 or rs1799782. In the present study, we found that Arg194Trp (rs1799782) polymorphism in XRCC1 is associated with GC occurrence. Our results are in agreement with a recent meta-analysis by Zhao et al. [20] based on 17 studies with a total population of 10427 participants, which suggested that for Arg194Trp polymorphism, mutant gene carriers had higher GC risk among Asians.

We note several limitations of the current study. First, we used 1244 subjects, which may be insufficient to demonstrate small risk effects. Second, we focused only on five non-synonymous variants of four DNA repair-relevant genes. Incorporating more functional variants of these proteins should provide a more comprehensive understanding. Finally, we did not explore whether associations between smoking, drinking, or *H. pylori* infections and polymorphisms existed because of unavailability of such data for the GC and control groups.

In summary, our findings provide evidence for a contributory role of XRCC1 rs1799782 and NQO1 rs1800566 polymorphisms in the occurrence of GC. Moreover, this study supports a logical role of DNA repair-relevant genes in the pathogenesis of GC.

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Disclosure of conflict of interest

None.

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