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Research paper

Association of genetic variations in IL-6/IL-6R pathway genes with gastric cancer risk in a Chinese population

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ABSTRACT

Interleukin-6 (IL-6) and its receptor (IL-6R) were regarded to be responsible for the occurrence of gastric cancer for their regulation roles in the inflammation. The genetic variations in these two genes (*IL-6*: rs6949149, rs1800796, rs10499563 and *IL-6R*: rs2228145) have been suggested to be associated with gastric cancer risk. However, the published results were inconsistent among subjects of different ethnicity. To evaluate such an association in Chinese population, we carried out this case-control study based on 473 patients with gastric cancer and 474 healthy controls, whose genotypes were detected by the Sequenom MassARRAY platform, and *Helicobacter pylori* infection was assessed by immunogold testing kit. This study showed that rs1800796 CG genotype was associated with decreased risk of gastric cancer (adjusted OR = 0.75, 95% CI: 0.57–0.99, $p = 0.043$). The stratified analysis revealed that, in the *Helicobacter pylori* negative infection subgroup, rs2228145 AC (adjusted OR = 0.64, 95% CI: 0.42–0.97) and AC/CC (adjusted OR = 0.66, 95% CI: 0.45–0.99) genotypes were associated with decreased risk of gastric cancer, respectively. In contrast, in the *Helicobacter pylori* positive infection subgroup, rs10499563 TC (adjusted OR = 0.64, 95% CI: 0.43–0.95), CC (adjusted OR = 0.35, 95% CI: 0.14–0.90), TC/CC (adjusted OR = 0.59, 95% CI: 0.40–0.87) genotype were associated with decreased gastric cancer risk, respectively. Moreover, in the male subgroup, rs1800796 CG (adjusted OR = 0.61, 95% CI: 0.44–0.84) and CG/GG (adjusted OR = 0.67, 95% CI: 0.49–0.91) genotype were associated with decreased risk of gastric cancer, respectively. In short, this study suggested that *IL-6R* rs2228145 and *IL-6* rs10499563 genotype were associated with decreased risk of gastric cancer for the individuals with negative and positive *Helicobacter pylori* infection.

1. Introduction

Gastric cancer is the fourth most common cancer worldwide. Population in Eastern Asia including China have a relatively high incidence rates, which may be due to the effect of dietary patterns, food storage, and the availability of fresh produce, and the prevalence of *Helicobacter pylori* (*H. pylori*) infection (Torre et al., 2015). Actually, genetic and environmental factors are two main groups of risks associated with the occurrence of gastric cancer, and the risk factors includes *H. pylori* infection (Gonzalez et al., 2013), dietary habit (Jakszyn and Gonzalez, 2006), smoking and drinking (Sjodahl et al., 2007). For genetic reason, inflammation is an essential part of the carcinogenic process in gastric cancer (Macarthur et al., 2004), indicating the genetic background of inflammatory genes was a risk of gastric cancer.

Interleukin-6 (IL-6) is a versatile cytokine produced by immune and other kinds of cells that play an important role in the inflammatory responser, endocrine and metabolic function regulator (Basso et al., 1996). IL-6 triggers intracellular signaling by binding to its receptor (IL-6R). Studies reported that up-regulated IL-6/IL6R system has shown a prognostic impact on patients with hematologic malignancies and with solid tumors (Lippitz, 2013). Previous study revealed that gastric cancer patients have increased levels of IL-6 in gastrointestinal cells and in the mucosa (Matsuo et al., 2003); moreover, IL-6 is also involved in the initiation, development, and prognosis of several cancers (Berger, 2004; Klampfer, 2011; Smith et al., 2001).

The human *IL-6* gene is located on chromosome 7p21, and the single nucleotide polymorphisms (SNPs) at promoter region of the *IL-6* gene has been identified to be associated with risk of gastric cancer (Wang et al., 2012; Wang et al., 2013; Yang et al., 2013; Yin et al., 2012);

Abbreviations: IL-6, Interleukin-6; IL-6R, Interleukin-6 receptor; SNPs, Single nucleotide polymorphisms; *H. pylori*, *Helicobacter pylori*; HWE, Hardy-Weinberg equilibrium; OR, odds ratios; CI, confidence intervals

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however, the results of such associations were conflicting and inconclusive, and the understanding of the contribution of *IL-6* polymorphisms to the gastric cancer risk remains obscure, which may be caused by ethnic differences, inadequate statistical power of small sample size, publication bias, interaction of polymorphisms and environmental factors or some other reasons.

Moreover, a misssense variant in *IL-6R* (rs2228145 A/C) causes an Asp358Ala amino acid substitution within the extracellular cleavage domain of the IL-6R causing proteolytic cleavage of the membrane-bound IL-6R, and the minor allele was observed as a strong inducer of the soluble form of the IL-6 receptor, causing an increase in IL-6 circulating levels (Ferreira et al., 2013). To date, the susceptibility of the polymorphism to gastric cancer is unclear. Hence, we conducted a case-control study in a Chinese population to investigate the association between polymorphisms in *IL-6/IL-6R* and gastric cancer risk.

2. Materials and methods

2.1. Subjects

A population-based case-control study was performed between 1999 and 2006 in Jiangsu province, China. For cases, a total of 473 gastric cancer patients received surgical resection of the tumor, which was diagnosed histologically as gastric cancer. Clinical characteristics of patients were collected from their medical records and structured interviews by using a questionnaire. The control series included 474 healthy subjects matching the age, sex, and the distribution of gastric cancer cases were enrolled from the general population of Jiangsu in eastern China. All the participants gave informed consent for participation in this study and the study protocol was approved by the Kunshan Hospital Affiliated to Nanjing University of Chinese Medicine.

2.2. Polymorphisms selection and genotyping

The selected polymorphisms information were presented in the Table 1. Three polymorphisms in the promote region of *IL-6* may have effect on the expression of IL-6, and one in the exon 9 of *IL-6R* cause the substitution (Asp358Ala). These polymorphisms have potential function on the *IL-6/IL-6R* pathway, and their minor allele frequencies (MAF) were above 0.05 in Chinese population. The genotyping of all polymorphisms were based on the sequenom MassARRAY platform, according to the standard protocol recommended by the manufacturer (Sequenom, Inc.). Multiplexed SNP MassEXTENDED assay was designed by Sequenom MassARRAY Assay Design 3.0 Software (Yin et al., 2012). Finally, data management and analysis were performed by Sequenom Typer 4.0 Software (Yin et al., 2012; Yu et al., 2011).

2.3. H. pylori serum detection

The *H. pylori* infection of all participants were detected with a commercial *H. pylori* immunogold testing kit (Kangmei Tianhong Biotech (Beijing) Co., Ltd., Beijing, China) according to the suggested procedures, which were validated in the Chinese populations with sensitivity of 98.29% and specificity of 98.51% for the detection of *H. pylori* infection.

Table 1
the information of the polymorphisms.

Gene	SNP ID	Chromosome	Allele	Position
IL6	rs6949149	7:22,709,538	T/G	− 17,724 T > G
IL6	rs10499563	7:22,720,869	T/C	− 6331 T > C
IL6	rs1800796	7:22,726,627	C/G	− 572C > G
IL6R	rs2228145	1:154,454,494	A/C	Intron, Asp358Ala

Table 2
Frequency distribution of demographic characteristics of gastric cancer cases and cancer-free controls.

	Controls, n (%)	Cases, n (%)	*p* value
Total participants	474	473	
Age (mean ± SD)	64.55 ± 11.90	64.63 ± 11.89	0.925[a]
Gender			
Male	343(72.36)	347(73.36)	0.730[b]
Female	131 (27.64)	126(26.64)	
Drinking			< 0.001[b]
Yes	19(4.01)	53(11.21)	
None	455(95.99)	420(88.79)	
Smoking			< 0.001[b]
Yes	61(12.87)	110(23.26)	
None	413(87.13)	363(76.74)	
Helicobacter pylori infection status			
Positive	232(48.95)	258(54.55)	0.085[b]
Negative	242(51.05)	215(45.45)	

[a] Independent *t*-test.
[b] Two-sided $\chi 2$ test for distributions between cases and controls.

2.4. Statistical analysis

Hardy-Weinberg equilibrium in the control group was tested using a goodness of fit chi-square test. The differences of genotype distribution were evaluated by Pearson's $\chi 2$ test. The associations between polymorphisms and gastric cancer risk were measured by adjusted odds ratios (ORs) with 95% confidence intervals (CIs), which were calculated by multivariate logistic regression with adjustments for gender, age and *H. pylori* infection status. All of the statistical analyses above were carried out by using SPSS 16.0 software (SPSS, Chicago, IL, USA). $p < 0.05$ for all two-sided tests was regarded as statistically significant.

3. Results

3.1. Characteristics of the study population

A total of 474 healthy individuals and 473 gastric cancer patients were enrolled for the controls and cases, respectively. The demographic and exposure data of all participants are summarized in Table 2. The mean age of cases and controls were 64.63 ± 11.89 and 64.55 ± 11.90 years old, respectively, and no significant difference was observed between the two groups($p = 0.925$). The gender distribution among the two groups was also no significant difference ($p = 0.730$). For the *H. pylori* infection, the positive ration in cases (54.55%) was higher than that in controls (48.95%) although no significant difference was exhibited between the two groups ($p = 0.085$). For the drinking and smoking, the ratio of individuals who have smoking and drinking habits was significant higher in cases than that in controls(Drinking: cases, 11.21% *vs.* controls, 4.01%, $p < 0.001$; Smoking: cases, 23.26% *vs.* control, 12.78%, $p < 0.001$).

3.2. Genotype distribution and the association of the polymorphisms and gastric cancer risk

The genotype distributions of the enrolled polymorphisms are summarized in Table 3. All tested genotypes of each polymorphism in controls did not deviate from Hardy-Weinberg equilibrium (rs2228145: $p = 0.959$; rs6949149: $p = 0.770$; rs1800796: $p = 0.750$; rs10499563: $p = 0.600$).

The association logistic regression analysis revealed that rs1800796 CG genotype was associated with decreased risk of gastric cancer (adjusted OR = 0.75, 95% CI: 0.57–0.99, $p = 0.043$). However, there was no significant association between any other polymorphism and gastric cancer risk.