RESEARCH ARTICLE

IL-6-6331 (T/C, rs10499563) is Associated with Decreased Risk of Gastric Cancer in Northern Chinese

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Abstract

Background: Polymorphisms of genes encoding cytokines could be potential biomarkers to predict risk of gastric cancer (GC). Here, we investigated the association between the *IL-6* -6331 (T/C, rs10499563) polymorphism in its promoter region and GC risk. Methods: In this case-control study of 215 GC cases and 518 non-cancer controls, the *IL-6* -6331 (T/C, rs10499563) polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: Individuals with the TC or CC genotype were associated with a significantly decreased risk of GC (OR=0.710, 95% CI: 0.504-0.999, *P*=0.049) compared with TT wild-type carriers. Ther C allele was also associated with significantly decreased risk of GC (OR=0.715, 95% CI: 0.536-0.954, *P*=0.023) compared with the T allele. In the stratification analysis, TC or CC genotypes were associated with significantly decreased GC risk in subgroups of males, people older than 60, and *H. pylori*-positive cases. However, no significant interaction was observed for TC or CC genotypes with *H. pylori* infection. On stratification with the Lauren classification, TC or CC genotypes were associated with significantly decreased risk of diffuse-type GC (OR=0.497, 95% CI: 0.266-0.925, *P*=0.027), also in subgroups of males, people older than 60, and *H. pylori*-positive cases. Conclusions: The *IL-6* -6331 (T/C, rs10499563) polymorphism is associated with genetic susceptibility of GC and may have the potential to predict GC risk.

Keywords: Gastric cancer - atrophic gastritis - *IL-6* - polymorphism - *H. pylori*

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Introduction

Genetic susceptibility plays a pivotal role in gastric carcinogenesis and may predict individuals with high risk of gastric cancer (GC) (Ponder, 2001). Interleukin 6 (*IL-6*) is a versatile cytokine which exerts essential functions in immune and inflammatory reactions under physiological conditions (Bauer et al., 1988). Under pathological conditions, *IL-6* could promote proliferation and inhibit apoptosis. In addition, *IL-6* has been reported to be involved in the occurrence and development of GC (Yu et al., 2009; Jarnicki et al., 2010).

IL-6 gene has polymorphisms which could influence the phenotype of host and further affect genetic susceptibility of cancer. Single nucleotide polymorphism (SNP) is the most common form of gene polymorphism and SNP within the promoter region of gene may influence the binding ability of transcriptional factor or the quality and quantity of the expression product of gene. Currently, studies mainly focused on -174 (G/C, rs1800795), -572 (G/C, rs1800796), -597 (A/G, rs1800797) polymorphisms in the promoter region of *IL-6* (Sugimoto et al., 2010; Yuzhalin, 2011). However, the results turned out to be controversial. A recent meta-analysis integrated the data and found that -174 (G/C, rs1800795), -572 (G/C, rs1800796), -597 (A/G, rs1800797) polymorphisms were not significantly associated with GC risk (Wang J et al., 2012; Yin YW et al., 2012). In addition, the frequencies of the polymorphisms in Asia and Latin America were 0%-9.8%, which could not be a suitable molecular biomarker to predict GC risk.

It has been reported that *IL-6* -6331 (T/C, rs10499563) polymorphism was a functional polymorphism site within the promoter region. *IL-6* -6331 polymorphism was first reported to be linked to disease in the acute infection stage of the operation of coronary artery reflux (Smith et al., 2008). It has been reported in 2011 that *IL-6* -6331 polymorphism was associated with GC risk in southern Chinese (Yu et al., 2011). As is known to all, *H. pylori* is one of the most important environmental factor in the stomach. However, the interactive effect of *IL-6* -6331 polymorphism with *H. pylori* has not been studied.

In this case-control study of northern Chinese, we investigated the association between *IL-6* -6331

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polymorphism and GC risk. Furthermore, the interaction of *IL-6* -6331 polymorphism with *H. pylori* and its association with risks of GC and its precancerous disease were also studied. The aim of this study was to reveal the basis of the difference of the GC risk among individuals with different *IL-6* genotypes and to provide experimental evidence for finding effective biomarker to predict risk of GC.

Materials and Methods

Study design and study population

The design of this study was approved by the Human Ethics Committee of China Medical University (Shenyang, China) before the outset of the research. The 733 individuals in this study were from people who underwent gastroscopy examination in the First Affiliated Hospital of China Medical University or the Central Hospital of Zhuanghe, Liaoning Province. The information of the individuals including sex, age and relevant clinical data was collected by questionnaires and medical records. Each individual involved in the study provided us with the written informed consents during epidemiological investigation. Finally, 733 individuals were included in this case-control study of 215 gastric cancer (GC), 189 atrophic gastritis (AG), 276 superficial gastritis (SG) and 53 normal people. The fasting venous blood was collected from each participant and was stored in -20 °C after centrifugation. The biopsy tissue from gastroscopy was diagnosed by histopathologic examination. The pathological examination was carried out by two professional pathologists and results of the diagnosis reached consensus. The superficial gastritis and atrophic gastritis were diagnosed according to Sydney classification. The normal individuals, SG and AG were regarded as non-cancer controls (518). The diagnosis of GC was performed according to WHO criteria and GC cases were classified by Lauren classification into 44 intestinal-type GC and 56 diffuse-type GC.

Genotyping of IL-6 -6331 polymorphism

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect *IL-6* -6331 (T/C, rs10499563) polymorphism. Genomic DNA of the blood samples from included subjects was extracted using routine phenol-chloroform method and stored at -20 °C. The primers of the PCR reaction were synthesized by Huada Gene Company. The forward primer was 5'-AGGGAAAGCAGGTTATCAAA-3' and the reverse primer was 5'-AGTGGC TTCA GGGAGACTAA-3'. The PCR reaction system included 10×PCR Buffer 2.5 μl, dNTP 2.0 μl, 10 pmol/μl forward primer and reverse primer 0.5 μl each, Taq DNA polymerase 0.2 μl, template DNA (10-100 ng) 1 μl and water for reaching totally 25 μl. The PCR reaction condition was 94 °C for 3 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 61 °C and 30 s at 72 °C, with a final elongation at 72 °C for 10 min. The amplified DNA fragment was 424 bp. The 5 μl PCR product was added tango Buffer 2.0 μl, restriction enzyme Taa I 0.5 μl and distilled water to 20 μl then digested at 37 °C overnight. The 5 μl PCR product was dyed by Genebuilder for 2 min and performed 2% agarose gel electrophoresis at 160 V, 45min. Approximately 10% of the three results of the samples were randomly selected for sequencing by Huada Gene Company.

H. pylori serology detection

The method for the examination of *H. pylori* serology has been described in detail in our previous study (Gong et al., 2010). *H. pylori* IgG antibody was measured by ELISA (Helicobacter pylori IgG kit; Biohit, Helsinki, Finland). An individual was considered positive if the readings were ≥34 enzyme immune units (EIU).

Statistical analysis

Pearson's χ^2 test was applied to evaluate the differences of genotype distribution. The adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) of the relation between *IL-6* -6331 polymorphism and GC risk were calculated by multivariate logistic regression with adjustments for gender, age and *H. pylori* infection status. Likelihood ratio test was performed to assess the interaction effects of genotype and *H. pylori* on the risk of GC by comparing the model only involving main effects of gender, age, *H. pylori* and genotype with the full model also containing the interaction term of genotype with *H. pylori*. 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.

Results

Baseline characteristics of the study population

The baseline characteristics of the study population in this study were presented in Table 1. As there were statistical differences of sex, age between case group and control group, the multivariate logistic regression was performed to investigate the relation between *IL-6* -6331 polymorphism and GC risk in the further analysis with adjustment of sex, age and *H. pylori* infection.

Identification of IL-6 -6331 polymorphism

The amplified DNA fragment was 424 bp (Figure 1A). After restriction enzyme reaction and agarose gel electrophoresis, the fragment length of TT genotype was 424 bp; the fragment lengths of CC genotype were 320

Table 1. The Basic Messages of the Study

Variability	Non-GC	GC		
		Total	Intestinal-type GC	Diffuse-type GC
All cases (n)	518	215	44	56
Age		P=0.022		
Mean±SD	56.17±11.68	58.32±11.28	59.64±10.90	57.41±10.97
Median	56	58	57	57
Range	27-87	27-83	27-80	34-82
Gender		P=0.027		
Male	327(63.1)	154(71.6)	29(65.9)	38(67.9)
Female	191(36.9)	61(28.4)	15(34.1)	18(32.1)
H.pylori		P=0.644		
Positive	238(46.0)	103(47.9)	38(67.9)	29(51.8)
Negetive	279(54.0)	112(52.1)	18(32.1)	27(48.2)

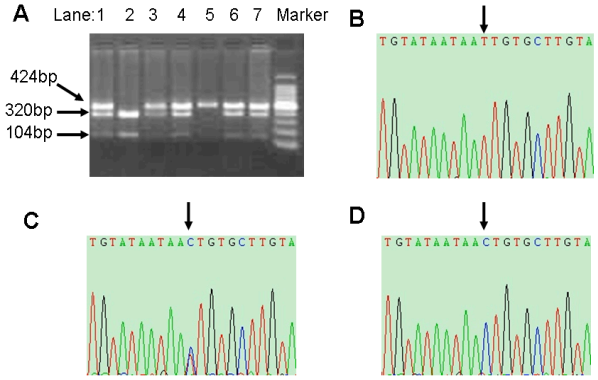

Figure 1. Agarose Gel Electrophoresis and Sequencing for *IL-6* -6331 T/C Polymorphism. A: agarose gel electrophoresis for 2% agarose at 160V, 45 min. Lane lane1, 3, 4, 6, 7: TC genotype; 2: CC genotype; 5: TT genotype; 8: 100bp Marker. B-D: The sequencing figure for different genotype of IL-6-6331 T/C polymorphism. B: TT genotype; C: TC genotype; D: CC genotype

Table 2. Association of IL-6 -6331 T/C Polymorphisms with the Risk of Gastric Cancer[a]

	Non-GC	GC	GC vs CON	
	n(%)	n(%)	*P* value	OR (95%CI)
TT	305(58.9)	141(65.6)		1.0(Ref)
TC	178(34.4)	67(31.2)	0.135	0.762(0.534-1.088)
CC	35(6.8)	7(3.3)	0.058	0.441(0.189-1.028)
TC+CC			**0.049**	**0.710(0.504-0.999)**
C vs. T			**0.023**	**0.715(0.536-0.954)**
P_{HWE}[b]	0.2			

[a]using Logistic Regession adusted by gender, age and *H.pylori* infection status; [b]means Hardy-Weinberg Equilibrium in population; Non-GC, cancer-free controls; GC, gastric cancer

bp and 104 bp; the fragment lengths of TC genotype were 424 bp, 320 bp and 104 bp. The PCR products of these three genotypes were sequencing to confirm the results (Figure 1B, C, D).

Association between IL-6 -6331 genotypes and GC risk

In this study sample, the distribution frequencies of TT, TC and CC genotypes in the control group were 8.9%, 34.4% and 6.8% respectively, which was in agreement with the Hardy-Weinberg equilibrium (HWE) (P=0.200). As a result, the population in this study was representative. Individuals with TC or CC genotype were associated with significantly decreased risk of GC (OR=0.710, 95%CI: 0.504-0.999, P=0.049) compared with TT wild-type carriers. C allele was also associated with significantly decreased risk of GC (OR=0.715, 95%CI: 0.536-0.954, P=0.023) compared with T allele (Table 2).

Stratification analysis of the association between IL-6 -6331 genotypes and GC risk

In the subgroup of people older than 60, individuals with TC or CC genotype were associated with significantly decreased risk of GC (OR=0.456, 95%CI: 0.216-0.797, P=0.006, Table 3) compared with TT wild-type carriers; in the subgroup of males, TC or CC genotype carriers were observed to be associated with significantly decreased risk of GC (OR=0.664, 95%CI: 0.444-0.994, P=0.047, Table 3); in the subgroup of *H. pylori* infection positive, TC or CC genotype carriers were observed to be associated with significantly decreased risk of GC (OR=0.608, 95%CI: 0.376-0.982, P=0.042, Table 3).

Table 3. Association of *IL-6* -6331 T/C Polymorphisms with the Risk of Gastric Cancer Stratified by Host Characteristics

Variables	Genotype	Non-GC	GC	*P*	OR(95%CI)
Age[a]					
≤60	TT	214(61.3)	78(61.4)		1(Ref)
	TC	111(31.8)	45(35.4)	0.672	1.099(0.710-1.701)
	CC	24(6.9)	4(3.1)	0.142	0.441(0.148-1.316)
	TC+CC			0.913	0.977(0.641-1.488)
	C vs. T			0.46	0.876(0.616-1.245)
>60	TT	91(53.8)	63(71.6)		1(Ref)
	TC	67(39.6)	22(25.0)	**0.011**	**0.467(0.260-0.838)**
	CC	11(6.5)	3(3.4)	0.166	0.390(0.103-1.477)
	TC+CC			**0.006**	**0.456(0.261-0.797)**
	C vs. T			**0.008**	**0.525(0.327-0.843)**
Sex[a]					
Male	TT	186(56.9)	102(66.2)		1(Ref)
	TC	118(36.1)	47(30.5)	0.127	0.721(0.474-1.097)
	CC	23(7.0)	5(3.2)	0.064	0.388(0.142-1.056)
	TC+CC			**0.047**	**0.664(0.444-0.994)**
	C vs. T			**0.022**	**0.673(0.479-0.945)**
Female	TT	119(62.9)	39(63.9)		1(Ref)
	TC	60(31.4)	20(32.8)	0.937	0.975(0.520-1.827)
	CC	12(6.3)	2(3.3)	0.36	0.486(0.103-2.279)
	TC+CC			0.714	0.893(0.488-1.635)
	C vs. T			0.507	0.814(0.505-1.402)
H.pylori[a]					
Negetive	TT	179(64.2)	75(67.0)		1(Ref)
	TC	80(28.7)	35(31.3)	0.916	1.026(0.633-1.665)
	CC	20(7.2)	2(1.8)	**0.048**	**0.224(0.051-0.990)**
	TC+CC			0.536	0.863(0.541-1.377)
	C vs. T			0.164	0.751(0.502-1.124)
Positive	TT	125(52.5)	66(64.1)		1(Ref)
	TC	98(41.2)	32(31.1)	**0.048**	**0.601(0.364-0.995)**
	CC	15(6.3)	5(4.9)	0.404	0.636(0.220-1.840)
	TC+CC			**0.042**	**0.608(0.376-0.982)**
	C vs. T			0.066	0.689(0.463-1.025)

[a]using Logistic Regession adusted by the other two factors of gender, age and *H.pylori* infection status; Non-GC, cancer-free controls; GC, gastric cancer

Table 4. The Interaction of *IL-6* -6331 T/C Polymorphisms and *H.pylori* with the Risk of Gastric Cancer

		GC vs Non-GC (n=214 Vs. 430)[a]	
		TC+CC	TT
H.pylori status			
Negetive	Case/Control	37/98	75/172
	OR(95%CI)	1	1.155(0.725-1.839)
Positive	Case/Control	37/79	66/81
	OR(95%CI)	1.241(0.720-2.136)	2.158(1.311-3.553)
		$P_{interaction}$=0.241	

[a]in order to analyze the interaction of *IL-6* -6331 T/C polymorphisms and H.pylori with the risk of gastric cancer, we matched the control group with GC, and P for age is 0.224, P for sex is 0.224, P for *H.pylori* is 0.009

Interaction analysis between IL-6 -6331 genotypes and H. pylori infection

Because it is observed in the stratification analysis that TC or CC genotype carriers were associated with significantly decreased risk of GC in *H. pylori* infection

Table 5. Association of *IL-6* -6331 T/C Polymorphisms with the Risk of Intestinal and Diffuse-type Gastric Cancer

Variables		Non-GC	Intestinal-type GC	Diffuse-type GC	Intestinal-type GC vs CON		Diffuse-type GC vs CON	
					P value	OR (95%CI)	*P* value	OR (95%CI)
All cases[a]	TT	305(58.9)	30(68.2)	41(73.2)		1		1
	TC	178(34.3)	13(29.5)	12(21.4)	0.261	0.674(0.339-1.340)	**0.031**	**0.475(0.242-0.934)**
	CC	35(6.8)	1(2.3)	3(5.4)	0.207	0.271(0.036-2.060)	0.438	0.615(0.180-2.101)
	TC+CC				0.14	0.605(0.311-1.180)	**0.027**	**0.497(0.266-0.925)**
	C vs. T				0.095	0.612(0.343-1.090)	**0.049**	**0.589(0.348-0.997)**
Stratified analysis[b]								
Age								
≤60	TT	214(61.3)	16(64.0)	25(69.4)		1		1
	TC	111(31.8)	9(36.0)	9(25.0)	0.903	1.055(0.448-2.487)	0.33	0.671(0.300-1.499)
	CC	24(6.9)	0(0.0)	2(5.6)	NA	NA	0.627	0.689(0.153-3.100)
	TC+CC				0.743	0.867(0.370-2.033)	0.289	0.667(0.316-1.411)
	C vs. T				0.408	0.730(0.347-1.538)	0.318	0.726(0.387-1.361)
>60	TT							
	TC	91(53.8)	14(73.7)	16(80.0)		1		1
	CC	67(39.6)	4(21.1)	3(15.0)	0.078	0.348(0.108-1.124)	**0.033**	**0.248(0.069-0.890)**
	TC+CC	11(6.5)	1(5.3)	1(5.0)	0.614	0.576(0.068-4.906)	0.545	0.518(0.061-4.367)
	C vs. T				0.074	0.373(0.127-1.101)	**0.03**	**0.284(0.091-0.888)**
Sex					0.129	0.493(0.198-1.228)	0.061	0.395(0.150-1.042)
Male	TT	186(56.9)	21(72.4)	29(76.3)		1		1
	TC	118(36.1)	7(24.1)	8(21.1)	0.135	0.504(0.206-1.237)	**0.038**	**0.419(0.184-0.954)**
	CC	23(7.0)	1(3.4)	1(2.6)	0.357	0.373(0.048-2.916)	0.215	0.275(0.036-2.119)
	TC+CC				0.089	0.477(0.203-1.119)	**0.02**	**0.393(0.179-0.863)**
	C vs. T				0.092	0.531(0.255-1.109)	**0.021**	**0.443(0.222-0.884)**
Female	TT	119(62.3)	9(60.0)	12(66.7)		1		1
	TC	60(31.4)	6(40.0)	4(22.2)	0.834	1.124(0.374-3.376)	0.446	0.631(0.194-2.059)
	CC	12(6.3)	0(0.0)	2(11.1)	NA	NA	0.618	1.514(0.297-7.710)
	TC+CC				0.92	0.945(0.317-2.821)	0.651	0.788(0.281-2.209)
	C vs. T				0.63	0.793(0.310-2.031)	0.957	0.978(0.428-2.232)
H.pylori								
Negetive	TT	180(64.3)	14(73.7)	20(74.1)		1		1
	TC	80(28.6)	5(26.3)	7(25.9)	0.307	0.626(0.255-1.536)	0.556	0.762(0.308-1.883)
	CC	20(7.1)	0(0.0)	0(0.0)	NA	NA	NA	NA
	TC+CC				0.365	0.614(0.214-1.765)	0.279	0.609(0.248-1.495)
	C vs. T				0.202	0.534(0.203-1.401)	0.131	0.531(0.234-1.207)
Positive	TT	125(52.5)	16(64.0)	21(72.4)		1		1
	TC	98(41.2)	8(32.0)	5(17.2)	0.63	0.771(0.267-2.226)	**0.018**	**0.295(0.107-0.812)**
	CC	15(6.3)	1(4.0)	3(10.3)	0.514	0.498(0.061-4.046)	0.794	1.193(0.317-4.486)
	TC+CC				0.258	0.608(0.256-1.441)	**0.045**	**0.418(0.178-0.982)**
	C vs. T				0.277	0.668(0.323-1.383)	0.194	0.634(0.319-1.261)

[a]using Logistic Regession adusted by gender, age and *H.pylori* infection status; [b]using Logistic Regression adusted by the other two factors of gender, age and *H.pylori* infection status; Non-GC, cancer-free controls; GC, gastric cancer

positive people, we performed interaction analysis of *IL-6* -6331 genotypes and *H. pylori* infection. However, no significant interactive effect was found ($P_{interaction}$=0.241, Table 4).

Association between IL-6 -6331 genotypes and risk of intestinal-type and diffuse-type GC

We further investigated the association between *IL-6* -6331 genotypes and risk of intestinal-type and diffuse-type GC and found that TC or CC genotype was associated with significantly decreased risk of diffuse-type GC (OR=0.497, 95%CI: 0.266-0.925, *P*=0.027, Table 5). In the intestinal-type GC, no significant association was found (*P*>0.05). In the stratified analysis, TC or CC genotype showed certain association with decreased risk of diffuse-type GC but no significant association was observed in intestinal-type GC. In subgroups of people older than 60, males, and *H. pylori*-positive people, TC or CC genotype was associated with significantly decreased risk of diffuse-type GC with corresponding ORs of 0.284, 0.393 and 0.418 (95%CI=0.091-0.888, 0.179-0.863, 0.178-0.982, respectively, Table 5).

Discussion

IL-6 -6331 polymorphism has been reported to be associated with GC risk in southern Chinese (Yu et al., 2011). In this study, we investigate, for the first time, the frequency distribution of *IL-6* -6331 polymorphism in northern Chinese. It is revealed that *IL-6* -6331 polymorphism was associated with GC risk especially in diffuse-type GC.

IL-6 is a versatile inflammatory cytokine and its function was related to its amount in the tissue. Normal

amount of *IL-6* benefits the body and overexpressed *IL-6* would cause a series of inflammatory damages. *IL-6* has been reported to be associated with various kinds of cancers including breast, ovarian, pancreatic, prostate, renal, colorectal, and gastric cancer (Waldner et al., 2012). Serum levels of *IL-6* are higher in patients with gastric cancer than gastritis (Crabtree et al., 1991). Furthermore, *IL-6* was associated with GC development and low survival after operation (Ashizawa et al., 2005; Liao et al., 2008).

The gene encoding cytokine *IL-6* is mapped to 7q21.3, which includes 5 exons and 4 introns. In the promoter region of *IL-6* gene, the most frequently studied polymorphisms in relation to GC risk were -174 (G/C, rs1800795), -572 (G/C, rs1800796) and -597 (A/G, rs1800797) (Sugimoto et al., 2010; Yuzhalin, 2011). As for the relation of *IL-6* -6331 polymorphism with GC risk, only one study reported that it was associated with GC risk in southern Chinese in which TC genotype was associated with lower risk of GC and this polymorphism was a protective factor (Yu et al., 2011). On the basis of this, the present study discussed the association of *IL-6* -6331 polymorphism with GC risk and its interaction with *H. pylori* in northern Chinese. It is revealed that individuals with TC or CC genotype were associated with significantly decreased risk of GC (OR=0.710, 95%CI: 0.504-0.999, *P*=0.049) compared with TT wild-type carriers. Smith et al reported that wild-type TT genotype patients who had coronary artery bypass grafting operation had higher level of *IL-6* while CC genotype patients had lower level of *IL-6*, which was confirmed by in vitro luciferase assay (Smith et al., 2008). These results could, at least in part, explain our findings. Superabundant *IL-6* would promote gastric carcinogenesis. According to Smith et al's results, the wild-type genotype of *IL-6* -6331 polymorphism generated higher level of *IL-6* and might be associated with increased risk of GC; the mutant-type genotype of *IL-6* -6331 polymorphism generated lower level of *IL-6* and might be associated with decreased risk of GC.

Our results also revealed that TC or CC genotype was associated with significantly decreased GC risk in subgroups of people older than 60, males, and *H. pylori*-positive people with ORs of 0.456, 0.664 and 0.608 respectively (All *P*<0.05). This may arise from different exposure to environmental factors and various immune statuses of different people. Males prefer unhealthy life styles such as drinking and smoking, which might induce more inflammatory factors; the decline of immunity and the accumulation of inflammation of older people would generate more inflammatory factors such as *IL-6*; *H. pylori* was related to inflammation, and chronic inflammatory reactions induced by *H. pylori* would increase the risk for GC (Sipponen et al., 2000). Similar studies have reported that the relation between gene polymorphism and cancer risk was more obvious in certain subgroup, which may result from different characteristic of the host (e.g. environmental factors and immune conditions) (Yang et al., 2011; He et al., 2012; Chen et al., 2013).

Studies have shown that in the process of *H. pylori* infection-related gastritis→ intestinal metaplasia→ intestinal-type GC, the interaction between polymorphisms of host genes (such as cytokines including interleukins) and *H. pylori* infection play an important role (Atherton, 2006). In this study, TC or CC genotype were associated with significantly decreased risk of GC in *H. pylori* positive subgroup but no significant association was found in *H. pylori* negative subgroup. We further investigated the interaction between *IL-6* -6331 polymorphism and *H. pylori* but no significant interaction was observed. It might be because that the effect of *IL-6* -6331 polymorphism on GC risk was relatively low than *H. pylori*.

It was also revealed in the present study that TC or CC genotype was associated with significantly decreased risk of diffuse-type GC. In the further stratification analysis, TC or CC genotype carriers were associated with significantly decreased risk of diffuse-type GC in subgroups of males, people older than 60, and *H. pylori*-positive people, which was in agreement with the stratification analysis of total study sample. Although the pathogenesis and morphogenesis of diffuse-type GC were largely unknown (Sipponen, 2002), the results of this study provided some clues to understand the occurrence of diffuse-type GC.

To be concluded, this case-control study of northern Chinese preliminarily investigated the association of *IL-6* -6331 polymorphism with GC risk and found that individuals with TC or CC genotype were associated with significantly decreased risk of overall GC and diffuse-type GC. In the stratification analysis, TC or CC genotype was significantly associated with decreased risk of overall GC and diffuse-type GC in subgroups of males, people older than 60, and *H. pylori*-positive people. However, no significant interaction of *IL-6* -6331 polymorphism with *H. pylori* was observed. This study initially revealed that *IL-6* -6331 (T/C, rs10499563) polymorphism was associated with risk of GC and diffuse-type GC in northern Chinese, which might have potential role in predicting the risk of GC.

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