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Correlation between Interleukin-17 gene polymorphism and gastric cancer susceptibility in Han Chinese population

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Abstract. – OBJECTIVE: The present study aims to investigate the correlation between Interleukin (IL)-17 gene polymorphism with gastric cancer susceptibility in Han Chinese population.

PATIENTS AND METHODS: Between November 2013 and October 2014, 386 patients with gastric cancer who had undergone surgeries at our institution and 374 age- and sex-matched healthy controls were included in this study. Single nucleotide polymorphisms (SNPs) of IL-17 gene (rs2275913, rs3748067, rs4711998 and rs763780) in patients and health controls were studied by using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) and DNA sequencing technology. The correlation between IL-17 polymorphism and gastric cancer susceptibility was analyzed using logistic regression analysis.

RESULTS: For IL17 rs2275913, no significant differences were observed in the frequencies of AA genotype and A allele between patients and controls (χ^2 = 0.870 p > 0.05; χ^2 = 0.814 p > 0.05). In IL17 rs3748067, the frequencies of TT genotype and T allele were significantly higher in patients than in controls (χ^2 = 12.82 p < 0.01; χ^2 = 12.805 p < 0.01). For IL17A rs4711998, no significant differences were observed in the frequencies of AA genotype and A allele between patients and controls (χ^2 = 2.636, p > 0.05; χ^2 = 1.462, p > 0.05). As for ILI7F rs763780, the frequencies of GG genotype and G allele in patients were significantly different from those in controls (χ^2 = 16.534, p < 0.01; χ^2 = 16.399, p < 0.01).

CONCLUSIONS: Polymorphism of IL-17 rs3748067 and rs763780 is closely associated with gastric cancer development. Polymorphism of L-17 rs2275913 and rs4711998 may be correlated with the risk for gastric cancer.

Key Words:
Single nucleotide polymorphism, RFLP-PCR, Gastric cancer, Interleukin-17.

Introduction

Interleukin-17 (IL-17), an IL-17 family member, is a proinflammatory cytokine secreted by activated CD4+ T helper (Th) 17 cells and neutrophils. IL-17 is an early initiation factor of inflammatory response by inducing the production and secretion of IL-6, IL-8 and other factors from epithelial cells, endothelial cells and fibroblasts. In addition, IL-17 is a main effector cytokine of Th17 cells and a potent proinflammatory cytokine by activating T cells and stimulating the production of multiple cytokines such as IL-6, IL-8, granulocyte macrophage colony stimulating factor (GM-CSF) and cellular adhesion molecule 1 (CAM-1) from epithelial cells, endothelial cells and fibroblasts, leading to the generation of inflammation. IL-17 exists as a homodimer with a molecular weight of 15 kiloDalton (kDa). IL-17, a polypeptide consisting of 155 amino acids, shows conservation in the c-termini

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by adopting a cysteine-knot formation. However, significant sequence variation is observed in the N termini which consists of 19-23 amino acid residues that are mainly related to signal transduction and share 72% homology with the open reading frame of herpes simplex virus (HSV 13) infecting squirrel monkeys. IL-17 family comprises six members including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F, among which, IL-17A and IL-17F are located at chromosome 6p12 and share 50% homology[1] whereas IL-17B, IL-17C and IL-17D share only 16%-30% homology. As IL-17A and IL-17F share greater homology, they are similar in terms of structure and function as well as regulation and signal transduction[2]. To this end, the present study investigated single nucleotide polymorphism (SNP) loci of IL-17A and IL-17F. Previous studies have shown that serial IL-17 significantly increases in autoimmune diseases such as multiple sclerosis (MS)[3], asthma[4], rheumatoid arthritis (RA)[5], systemic lupus erythematous[6] and allograft rejection[7]. Moreover, IL-17 expression is significantly elevated in inflammatory bowel disease, HP infection, hepatitis B virus infection, prostatitis of unknown etiology and tumor-related inflammation[8].

Gene polymorphism refers to at least two or more discrete genotypes or alleles simultaneously or frequently occurring in same population. Polymorphism is generated at the level of gene variation which commonly occurs in non-coding regions and gene regions harboring no significant regulatory functions with gene frequency more than 1%. However, mutation with gene frequency less than 1% which occurs in protein-coding regions or regulatory regions, causes hereditary differences between individuals, leading to the presence of same or different phenotypes[9,10]. Polymorphism comprises single nucleotide sequence variant, different copy numbers of repeated sequence and conversion or replacement between alleles, of which, SNPs is the most common variation and study of SNPs is an important strategy to investigate the relationship between genes and diseases.

Many studies have shown that chronic inflammation is closely associated with tumor. With the discovery of the inflammatory pathway of IL-23→Th17→IL17A/IL17F, IL-17, as a novel pro-inflammatory cytokine, has drawn increasing attention. Due to its specific binding to its receptor, IL-17 plays important roles in immune response and pro-inflammatory response. Currently, more studies have been focused on the relationship between IL-17 and diseases whereas fewer studies have been conducted on the polymorphism of IL-17. Relevant researches have only been reported in a few studies on chronic inflammatory diseases (such as asthma, RA, inflammatory bowel diseases, etc.). In the present study, SNPs loci of IL-17 were studied and compared between gastric cancer patients and normal controls by using restriction fragment length polymorphism - polymerase chain reaction (RFLP-PCR) and DNA sequencing technology to investigate the relationship between polymorphism of IL-17 and the genetic susceptibility to gastric cancer, providing important evidences for advancing the understanding of the pathogenesis of the disease at the level of genetics.

Patients and Methods

Patients

Between November 2013 and October 2014, 386 patients (males 221, females 165) who were confirmed pathologically with gastric cancer and had undergone surgeries at the Department of Gastrointestinal Surgery of Hebei Provincial People's Hospital, China were included in this study. Patients had received neither radio- and chemo-therapy before surgery or oral antibiotics two weeks prior to surgery. In addition, 374 age- and sex-matched healthy subjects (males 219, females 155) seeking for health check-up at the same institution were included as control group.

The present study was approved by the Ethics Committee of Hubei Provincial People's Hospital. Written informed consent was obtained from patients and controls. Clinical data of patients were obtained from questionnaires and medical records. No kinship was observed between subjects.

Sample Collection

A 4 ml fasting venous blood sample was collected from every subjects in EDTA anticoagulated tubes and stored at -80°C for DNA isolation.

DNA Isolation

Genomic DNA was isolated from peripheral blood samples using TIANamp Blood DNA kit (Tiangen Biotech (Beijing) Co., Ltd., China) by following the following steps.

1. A 200 µl anticoagulated blood sample was lysed in two volumes of CL and centrifuged at 10000 rpm for 1 min. Supernatant was aspirated and the cell pellet was mixed with 200 µl GS.
2. The above lysate was mixed with 20 µl Oroteinase K, resolved in 200 µl GB and placed at 56 °C for 10 min followed by mixing with 200 µl ethanol.
3. The resulting solution was loaded in a CB3 adsorption column, which was placed in a collection tube and centrifuged at 12000 rpm for 30 sec. The waste was discarded and the CB3 column was placed back into the collection tube.
4. A 500 µl of GD buffer was loaded in CB3 column and centrifuged at 12000 rpm for 30 sec. The resulting waste was discarded and the CB3 column was placed back into the collection tube.
5. A 600 µl PW buffer was loaded in CB3 column ad centrifuged at 12000 rpm for 30 sec. The resulting waste was discarded and the CB3 column was placed back into the collection tube.
6. Step 5 was repeated.
7. CB3 column was centrifuged at 12000 rpm for 2 min and the resulting waste was discarded. CB3 column was placed at room temperature for several minutes to dry out liquid residue in the column.
8. The CB3 column was transferred to a 1.5 ml centrifuge tube and 100 µl elution TB buffer was added to the adsorption membrane. The column was placed at room temperature for 5 min and centrifuged at 12000 rpm for 2 min. The resulting solution was collected into another centrifuge tube and stored at -20 °C.

DNA Concentration and Purity Determination

A 5 µl DNA sample was diluted to 1 ml in deionized water. SmartSpec 3000 DNA/RNA/protein spectrophotometer (BioRad Laboratories Ltd, Oakland, CA, USA) was zeroed with 1 ml of deionized water. Sample DNA solution was loaded in the spectrophotometer and the absorbance was read at 260 nm and 280 m. The DNA concentration and purity were calculated by using the following formula: DNA concentration = OD260 × 200 × 50 (µg/ml); DNA purity = OD260/OD280.

Genotyping of Polymorphism

PCR Primer Design

PCR primers were designed using Primer 5.0 software according to IL-17 gene sequence in GenBank and synthesized by Shanghai Sangon Biotech, China (Table I).

PCR Reaction Mixture: (a Total Volume of 20 µl)

Components	Volume
DNA template	1 µl
Primers:	
Forward primer (25 pmol/µl)	1 µl
Reverse primer (25 pmol/µl)	1 µl
dNTP mixture (10 nM, Promega, USA)	1 µl
10 × Pfu Buffer	2 µl
KOD plus (Pfu DNA polymerase, 5 U/µl, TOYOBO Co. Ltd., Japan)	0.5 µl
Deionized water	13.5 µl
Total	20 µl

Table I. The list of different genotypes primer of IL-17.

SNP	Enzyme	Recognition sequences	RFLP(bp)	Primer
368, 246, 122	GAANN/NNTTC	XmnI	rs2275913	Forward: 5′-AAGAAAGATCAAATGGAA-3′ Reverse: 5′-TCTCTCTTTTTATAGGGC-3′
358, 230, 128	R/AATTY	ApoI	rs3748067	Forward: 5′-TATGGGGAAAATGAAACC-3′ Reverse: 5′-GTAGGGCAAGACAGCACA-3′
382, 206, 176	T/CGA	TaqI	rs4711998	Forward: 5′-TTACACTCCAGCCATTGAGTTG-3′ Reverse: 5′-TGAAAATGGGGATAGAGACTGG-3′
346, 194, 152	CATG/	NIaIII	rs763780	Forward:5′-CTGGGAGGCAAAGTGCCG-3′ Reverse: 5′-AAGCTTTAATTGGACAAA-3′

PCR Reaction Conditions

The loci of IL-17 sequence including rs2275913, rs3748067, rs4711998 and rs763780 were amplified under the reaction conditions described in the study of Luya Wang[11] with the above PCR reaction mixture using Mastercycler® gradient PCR system (Eppendorf, Hamburg, Germany) (Table II).

PCR Products Identification

PCR products were separated by using 2% agarose (Ameresco, Framingham, MA, USA) gel electrophoresis with ethidium bromide (Promega, Madison, WI, USA) staining and resulting DNA fragments were identified by using HITACHI UV-330 UV-visible spectrophotometry (Hitachi, Tokyo, Japan).

RFLP-PCR

Reaction components	Volume
PCR products	
Restriction endonuclease (Xsp I, ClaI, Hinf I)	5 µl
(Takara, Japan)	1 µl
Buffer (Amresco Inc., USA)	2 µl
Deionized water	12 µl
Total	20 µl

PCR products were digested overnight and the reaction was terminated with 2 µl loading buffer. The digested fragments were separated using 8% PAGE-Gel electrophoresis performed at 100 mv for 50 min and identified using UVPGDS-8000 gel documentation system (UVP Inc. Upland, CA, USA).

Statistical Analysis

Statistical analyses were performed using SPSS software version 19.0 (SPSS Inc., Chicago, IL, USA). Differences in serological results between patients and controls were analyzed using *t*-test. Differences in gene frequencies and allele frequencies between patients and controls were analyzed using χ^2-test. Logistic regression analysis was performed to analyze the correlation between IL-17 polymorphism and gastric cancer susceptibility. p-value < 0.05 was considered statistically significant.

Results

General Conditions

No significant differences were observed in gender, age, blood lipid, blood glucose and smoking history between patients and controls (p > 0.05). However, significant difference was observed in drinking habits between two groups (Tables III, IV).

DNA Isolation

Genomic DNA was isolated and purified with high purity (OD260/OD280≈1.75), stable quality and intact fragment, satisfying the requirement for PCR and RFLP analysis.

RFLP-PCR Results

As for IL-17A (rs2275913), PCR products digested with XmnI restriction enzyme should yield two fragments sizes of 246 bp and 122 bp if this locus harbors GG genotype, three fragments of 368 bp, 246 bp and 122 bp in case of GA genotype and one fragment of 368 bp in case of

Table II. Procedures of touch-down PCR.

Response procedures	Degeneration		Annealing		Extend		Cycles
	Tm (℃)	Time (s)	Tm (℃)	Time (s)	Tm (℃)	Time(s)	
Predegeneration	95	300					
Main	95	40	68	60	72	45	3
Cycle	95	40	65	60	72	45	3
	95	40	63	60	72	45	3
	95	40	61	60	72	45	3
	95	40	60	60	72	45	3
	95	40	58	60	72	45	3
	95	40	57	60	72	45	3
	95	40	56	60	72	45	3
	95	40	54	60	72	60	20
Extend	72	300					

Table III. Baseline characteristics of cases and controls.

Characteristics	Cases (n = 386)		Controls (n = 374)		χ^2	*p*-value
	n	%	n	%		
Median age	59.8±11.3		58.3 ± 12.1			
Gender						
Male	221	57.25	219	58.56		
Female	165	42.75	155	41.44	0.132	0.71637
Tobacco smoking						
Yes	202	52.33	198	52.94		
No	184	47.67	176	47.06	0.028	0.86711
Alcohol consumption						
Yes	274	70.98	234	62.57		
No	112	29.02	140	37.43	6.073	0.01373

Table IV. The levels of BMI, TC, TG, HDL-C, GLU in different groups.

Group	N	BMI (kg/m^2)	Waist circumference (cm)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	GLU (mmol/L)
Cases	386	23.6 ± 2.4	85.2 ± 10.8	5.1 ± 0.8	1.0 ± 0.3	0.9 ± 0.4	5.1 ± 0.6
Controls	374	24.1 ± 2.8	83.4 ± 8.6	4.2 ± 0.7	1.1 ± 0.2	1.2 ± 0.3	4.9 ± 0.7

AA genotype. The results showed AA for sample 1, GA for sample 2, 3, 6 and 7 and GG for sample 4 and 5 (Figure 1).

As for IL-17A (rs3748067), PCR products digested with ApoI restriction enzyme should yield two fragments sizes of 230 bp and 128 bp in the presence of homozygous T allele, three fragments size of 358 bp, 230 bp and 128 bp in case of TC genotype and one fragment of 358 bp in case of CC genotype. The results demonstrated TC genotype for sample 1 and 5, TT genotype for sample 2 and 4, and CC genotype for sample 3 and 6 (Figure 2).

For sequence of IL-17A (rs4711998), PCR products digested with TaqI restriction enzyme should yield two fragments of 206 bp and 176 bp in the presence of GG genotype, GA genotype of 382 bp, 206 bp and 176bp in the presence of GA genotype, and one fragment of 382 bp in the presence of AA genotype. The results showed AA genotype for sample 1 and 3, GG genotype for sample 2 and 4, and GA genotype for sample 5, 6 and 7 (Figure 3).

As for IL-17A (rs763780), PCR products digested with NIaIII should yield two fragments of 194 bp and 152 bp in the presence of AA geno-

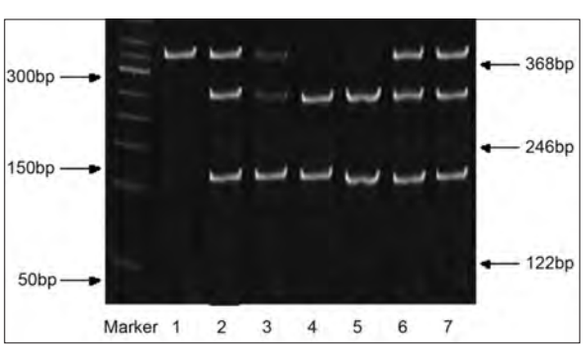

Figure 1. Electrophoretogram of XmnI enzyme digested in the RS2275913 of IL-17 gene. *Note:* GG genotype: 4, 5; AA genotype: 1; GA genotype: 2, 3, 6, 7.

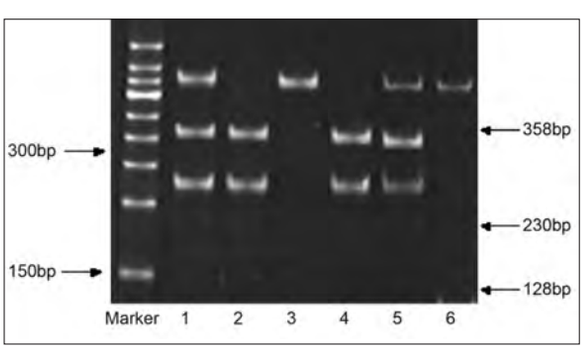

Figure 2. Electrophoretogram of ApoI enzyme digested in RS3748067 of IL-17 gene. *Note:* CC genotype: 3, 6; TT genotype: 2, 4; TC genotype: 1, 5.

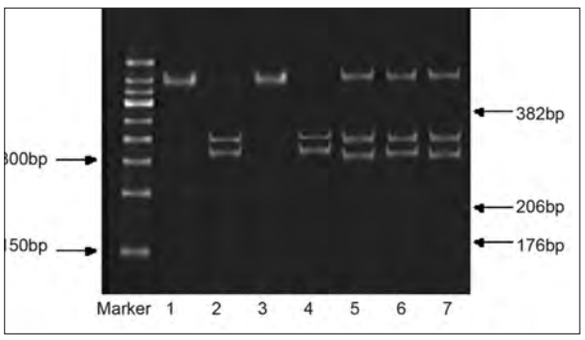

Figure 3. Electrophoretogram of TaqI enzyme digested in rs4711998 of IL-17 gene. *Note:* AA genotype: 1, 3; GG genotype: 2, 4; GA genotype: 5, 6, 7.

type, three fragments of 346 bp, 194 bp and 152 bp in the presence of AG genotype, and one fragment of 346bp in the presence of GG genotype. The results showed GG genotype for sample 1, AA genotype for sample 2 and 3, and GA genotype for sample 4 and 5 (Figure 4).

Genotype Distribution for Different Loci of IL17

For IL17 rs2275913, frequencies of genotypes of GG, GA and AA were 51.81%, 33.16% and 15.03%, respectively in patient group, and 54.28%, 32.89% and 12.83%, respectively in control group. In addition, G allele frequency was 68.39% in patients and 70.72% in controls. A allele frequency was 31.61% in patients and 29.28% in controls. No significant differences were observed in the frequencies of AA genotype and A allele between patients and controls ($\chi^2 = 0.870$ $p > 0.05$; $\chi^2 = 0.814$ $p > 0.05$) (Table VI).

For IL17 rs3748067, the frequencies of CC, CT and TT genotypes were 79.79%, 13.47% and 6.74%, respectively in patients, and 86.36%, 12.03% and 1.61% in controls. The frequency of C allele was 86.53% in patients and 92.38% in controls. T allele frequency was 13.47% in patients and 7.62% in controls. The frequencies of TT genotype and T allele were significantly higher in patients than in controls ($\chi^2 = 12.82$ $p < 0.01$; $\chi^2 = 1$ 2.805 $p < 0.01$) (Table VII).

For IL17A rs4711998, the frequencies of GG, GA and AA genotypes were 33.16%, 44.56% and 22.28%, respectively in patients and 36.10%, 46.52%, 17.38% in controls. The frequency of G allele was 55.44% in patients and 59.36% in controls. The frequency of A allele was 44.56% in patients and 40.64% in controls. No significant differences were observed in the frequencies of AA genotype and A allele between patients and controls ($\chi^2 = 2.636$, $p > 0.05$; $\chi^2 = 1.462$, $p > 0.05$) (Table VIII).

As for IL17F rs763780, the frequencies of polymorphism in patients were AA 76.16%, AG 15.03%, GG 8.81%, and AA 83.42%, AG 14.44%, GG 2.14% in controls. The frequency of A allele was 83.68% in patients and 90.64% in controls. The frequency of G allele was 16.32% in patients and 9.36% in controls. The frequencies of GG genotype and G allele in patients were significantly different from those in controls ($\chi^2 = 16.534$, $p < 0.01$; $\chi^2 = 16.399$, $p < 0.01$) (Table IX).

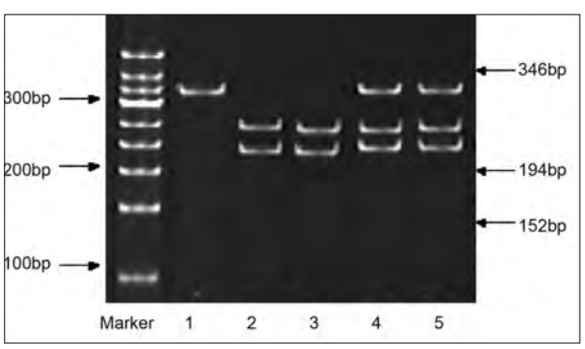

Figure 4. Electrophoretogram of NIaIII enzyme digested in RS763780 of IL-17 gene. *Note:* GG genotype: 1; AAgenotype: 2, 3; GA genotype: 4, 5.

Linear Regression Analysis With Binary Variable

Linear regression model analysis was performed taking gastric cancer as dependent variable and the CC, CT, TT genotypes in IL-

Table V. The serum levels of TNF-α, IL-6, Hs-CRP, IL-17, CEA in different groups ($\bar{x} \pm S$).

Group	N	TNF-α (pg/ml)	IL-6 (pg/ml)	Hs-CRP (mg/L)	IL-17 (pg/ml)	CEA (ng/ml)
Cases	386	10.23 ± 1.68	5.98 ± 0.42	4.24 ± 1.86	60.56 ± 11.68	9.41 ± 2.42
Controls	374	2.02 ± 0.75	0.54 ± 0.28	0.74 ± 0.18	48.97 ± 7.32	2.4 ± 0.67

Table VI. Distribution of locus genotype and allele frequency of IL-17 rs2275913.

Group	N	Genotype (%)				Allele (%)	
		GG	GA	AA	GA+AA	G	A
Cases	386	200 (51.81)	128(33.16)	58 (15.03)	186 (48.19)	528 (68.39)	244 (31.61)
Controls	374	203 (54.28)	123(32.89)	48 (12.83)	171(45.72)	529 (70.72)	219 (29.28)
χ^2		0.116	0.870	0.463		0.814	
p-value			0.73	0.35	0.50		0.37

Table VII. Distribution of locus genotype and allele frequency of IL-17 rs3748067.

Group	N	Genotype (%)				Allele (%)	
		CC	CT	TT	CT+TT	C	T
Cases	386	308 (79.79)	52 (13.47)	26 (6.74)	78 (20.21)	668 (86.53)	104 (13.47)
Controls	374	323 (86.36)	45 (12.03)	6 (1.61)	51 (13.64)	691 (92.38)	57 (7.62)
χ^2		0.774	12.82	5.82		12.805	
p-value			0.38	< 0.001	0.02		< 0.001

Table VIII. Distribution of locus genotype and allele frequency of IL-17 rs4711998.

Group	N	Genotype (%)				Allele (%)	
		GG	GA	AA	GA+AA	G	A
Cases	386	128 (33.16)	172 (44.56)	86 (22.28)	258 (66.84)	428 (55.44)	344 (44.56)
Controls	374	135 (36.10)	174 (46.52)	65 (17.38)	239 (63.90)	444 (59.36)	304 (40.64)
χ^2		0.065	2.636	0.723		1.462	
p-value			0.80	0.10	0.40		0.23

Table IX. Distribution of locus genotype and allele frequency of IL-17 rs763780.

Group	N	Genotype (%)				Allele (%)	
		AA	AG	GG	AG+GG	A	G
Cases	386	294 (76.16)	58 (15.03)	34 (8.81)	92 (23.84)	646 (83.68)	126 (16.32)
Controls	374	312 (83.42)	54 (14.44)	8 (2.14)	62 (16.58)	678 (90.64)	70 (9.36)
χ^2		0.405	16.534	6.191		16.399	
p-value			0.53	< 0.001	0.01		< 0.001

17rs3748067 as independent variable by using the following formulas: y = 0.531 (TT) + 0.235 (CT) + 0.895. For IL-17rs763780, the frequencies of AA, AG and GG genotypes were took as independent variables to be analyzed using the following formula: y = 1.102 (GG) + 0.635 (AG) + 3.135. The results were listed in Table X and XI.

Discussion

Gastric cancer is the second most common malignant tumor in China. It is a life-threatening malignancy with high incidence and poor prognosis.

The pathogenesis of gastric cancer is associated with multiple factors, including environmental factor, diet, genetic factor, immune factors, in-

Table X. Binary regression with gastric as dependent variable in IL-17 rs3748067.

Variables	Regression coefficient	Standard error	Normalization factor	t	*p*
(Constant)	0.895	0.321		16.27	0.00
TT	0.531	0.153	0.328	2.377	0.00
CT	0.235	0.087	0.195	3.21	0.01

Table XI. Binary regression with gastric as dependent variable in IL-17 rs763780.

Variables	Regression coefficient	Standard error	Normalization factor	t	*p*
(Constant)	3.135	0.175		3.51	0.00
GG	1.102	0.069	0.458	6.426	0.00
AG	0.635	0.042	0.129	2.486	0.00

fection and inflammation. In recent years, inflammatory factors have been increasingly considered to be correlated with the pathogenesis of gastric cancer. Chronic inflammation has been gradually accepted as the driven factor for tumors[12]. IL-17 family is a newly found family of several cytokine members, which are considered to be associated with the development and progression of tumors by either inhibiting the occurrence or promote the development of tumors. Upon the occurrence of acute inflammation, IL-17 is rapidly secreted and accumulated at the site of inflammation, destroy or eliminate foreign carcinogens and protect host from damage. Conversely, IL-17 can accelerate the progression of chronic diseases, in particular, accelerate the progression of tumors.

IL-17, a specific cytokine secreted from Th17 cells, can promote cell proliferation and angiogenesis by recruiting neutrophils that release inflammatory cytokines, thereby inducing the development of disorders such as chronic inflammation and tumors. A number of studies have shown that high level of IL-17 plays an important role in the pathogenesis and development of tumors. Meanwhile, more attention has been drawn to the role of IL-17 polymorphism in the development of tumors due to regulated transcription of this gene.

In addition to its correlation with several non-tumor disorders such as asthma, RA, inflammatory bowel disease, MS and periodontitis, studies have shown that SNP of IL-17 is closely associated with several tumors including cervical can-

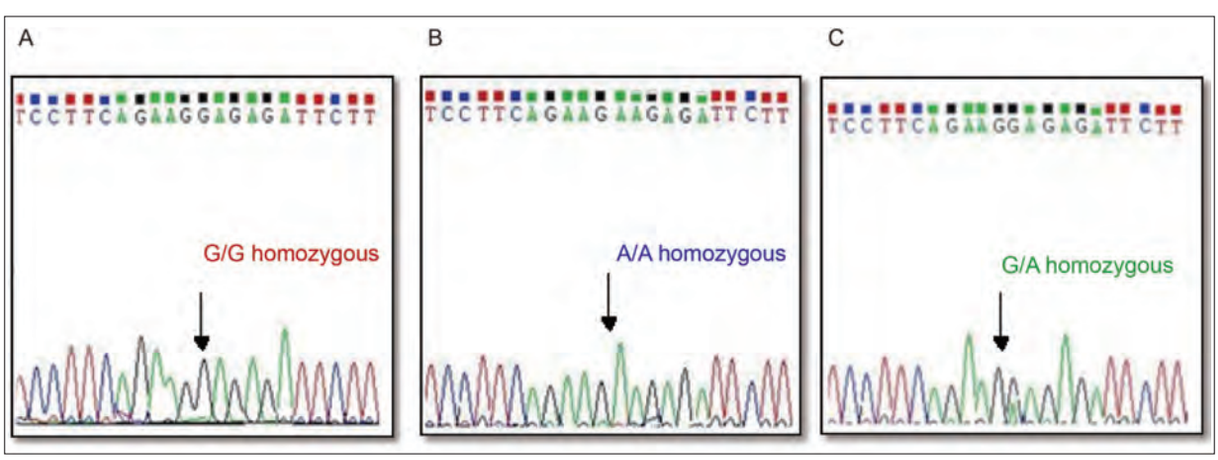

Figure 5. The partial DNA sequencing results of rs2275913 polymorphism in IL-17 gene, ***A,*** is G/G Homozygous; ***B,*** is A/A Homozygous; ***C,*** is G/A Homozygous.

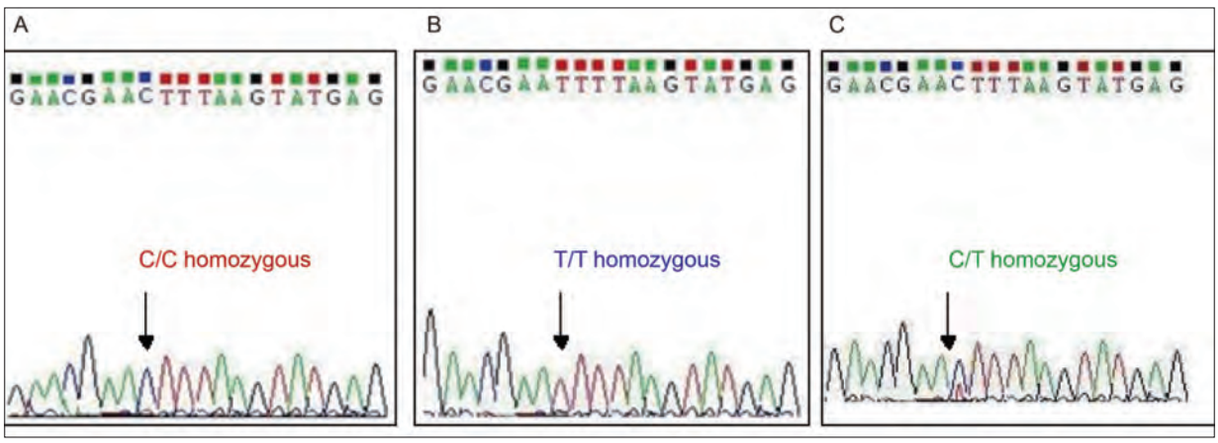

Figure 6. The partial DNA sequencing results of rs3748067 polymorphism in IL-17 gene, ***A,*** is C/C Homozygous; ***B,*** is T/T Homozygous; ***C,*** is C/T Homozygous.

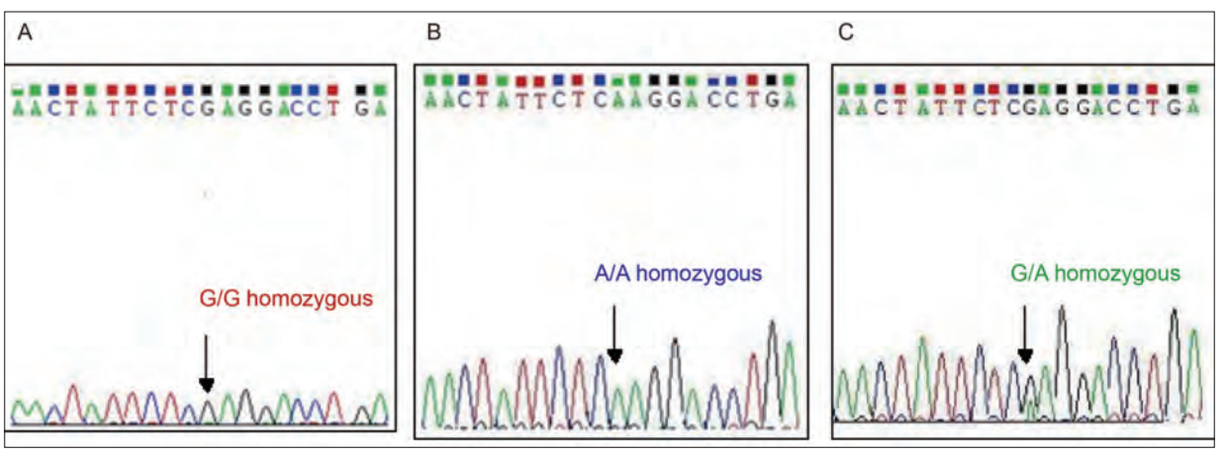

Figure 7. The partial DNA sequencing results of rs4711998 polymorphism in IL-17 gene, ***A,*** is G/G Homozygous; ***B,*** is A/A Homozygous; ***C,*** is G/A Homozygous.

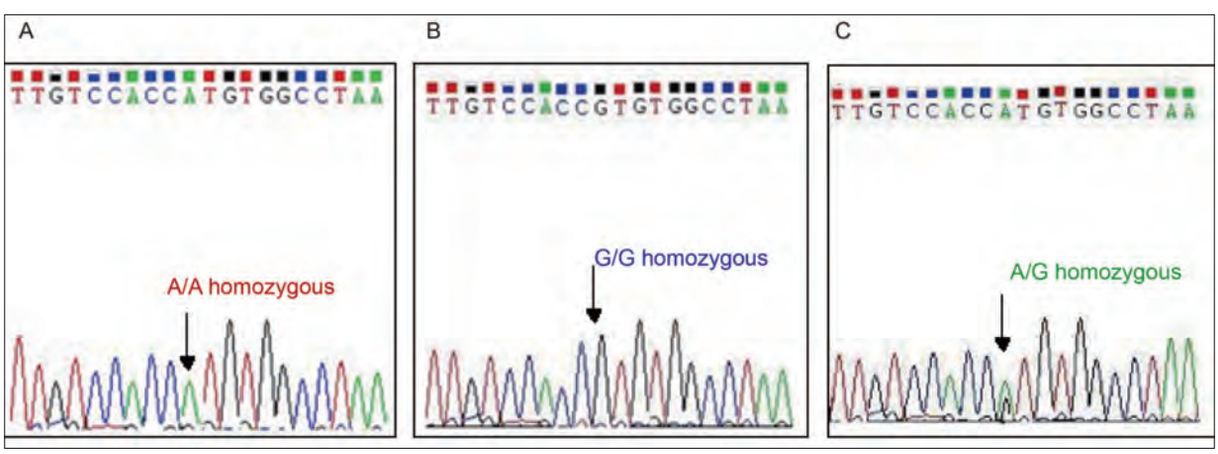

Figure 8. The partial DNA sequencing results of rs763780 polymorphism in IL-17 gene, ***A,*** is A/A Homozygous; ***B,*** is G/G Homozygous; ***C,*** is A/G Homozygous.

cer, lung cancer, colorectal cancer and breast cancer. However, few studies have been reported on the correlation between IL-17 polymorphism and gastric cancer. To this end, the correlation between IL-17 polymorphism and gastric cancer susceptibility in 386 patients admitted to our institution was investigated in the present study.

These results showed that no significant correlation was present between Il-17 polymorphism and gender or age, which is consistent with the result reported by Wu et al[13]. In addition, no significant correlation was observed between IL-17 polymorphism and BMI, waist circumference, blood lipid or blood glucose.

Controversial results have been obtained on the role of SNP of IL-17 rs2275913 in tumor development. This locus is located in the promoter region of this gene. Shibata et al[14] have reported that polymorphism of IL-17 rs2275913 is associated with gastric cancer development; in particular, frequency of AA homozygote is significantly high in intestinal type gastric cancer. Moreover, A allele is significantly correlated with the development of gastric cancer and increases the risk for the development of gastric atrophy. On the contrary, Wu et al[13] have demonstrated that the frequencies of IL-17 rs2275913 genotypes and alleles are not significantly different between gastric cancer patients and controls. In addition, AA homozygote is not correlated with the development of gastric cancer. We showed that frequencies of AA genotype and A allele in patients were not significantly different from those in controls, which is consistent with the result reported by Wu et al[13] but is inconsistent with Shibata et al[1]. The potential reason underlying this discrepancy is the difference in mutations between various ethnic groups in different population investigated.

Mutation of IL-17 rs763780 causes histidine-to-arginine substitution, which affects peptide synthesis and leads to the alteration in protein structure and function. Niu et al[15] showed that IL-17 rs763780 polymorphism is a potential risk factor for gastric cancer development and CC genotype significantly increases the risk for gastric cancer. Zhang et al[16] found that mutation at this gene locus significantly increases the development of gastric cancer in the population investigated and interacts with *H. pylori* infection, which is consistent with the results obtained in this study. However, Tahara et al[17] showed that polymorphism of IL-17 rs763780 is correlated neither with the pathogenesis and development of gastric cancer nor with CpG island hypermethylation. The study of Lanqing Bi also showed that polymorphism of this gene locus is not associated with gastric cancer development in population in Suzhou, China. However, both TC and CC genotypes increase the risk for gastric cancer susceptibility. Therefore, C allele might be correlated with gastric cancer development in this area but without statistical difference. These findings suggested that discrepancy in the results is attributed to the difference in the regions where the samples were collected. Therefore, increase in the sample size across geographic regions is required for further study.

IL-17 rs3748067, located at 3′UTR region, exert its function by regulating gene expression, gene stability and degradation as well as by controlling mRNA subcellular localization[18]. Zhang et al[16], Qinghai et al[19] and Costa et al[20] have showed that TT genotype at IL-17 rs3748067 significantly increases the risk for gastric cancer, which is consistent with the findings in the present study and those reported in previous studies. These findings suggest that mutation of IL-17 rs3748067 affects IL-17 protein expression and, thereby, increases susceptibility to gastric cancer.

Currently, some studies have been reported on the correlation of gene mutation of IL-17 rs4711998 with diseases. Chen et al[21] have showed that GG homozygote at this gene locus is closely associated with asthma development and asthma susceptibility in children. However, Fang have reported that polymorphism at this gene locus is not correlated with myocardial infarction. In addition, Yin et al[22] have showed that polymorphism of rs4711998 is significantly correlated with the susceptibility to esophageal cancer. Stratified analysis found that GA genotype significantly reduces the risk for esophageal cancer in non-smoking and non-alcoholic patients and young patients. To this date, no study is reported on the correlation between gene mutation at this locus and gastric cancer. The present study identified G→A mutation at rs4711998 locus. No significant differences were observed in frequencies of AA genotype and A allele between patients and controls (0.2228 *vs.* 0.1738, 0.4456 *vs.* 0.4064). However, this study has several limitations. Firstly, sampling in a specific region is not able to reflect the situation in other regions. Secondly, results are restricted by the sample size. Therefore, increase in sample size is required in further study. Furthermore, multicenter and multiethnic study is warranted to further confirm the

results of present study. Moreover, linear regression analysis will be performed using gastric cancer as dependent variable and mutation genotypes of IL-17 as independent variables to further confirm the correlation between polymorphism at rs3748067 and rs763780 and gastric cancer.

Conclusions

Polymorphism of IL-17 rs3748067 and rs763780 is closely associated with gastric cancer development. Polymorphism of L-17 rs2275913 and rs4711998 may be correlated with the risk for gastric cancer.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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