Int J Clin Exp Pathol 2015;8(6):7378-7384
www.ijcep.com /ISSN:1936-2625/IJCEP0009311

Original Article

Interleukin-17A gene polymorphism is associated with susceptibility to gastric cancer

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Received April 19, 2015; Accepted May 29, 2015; Epub June 1, 2015; Published June 15, 2015

Abstract: We conducted a study to investigate the role of three common SNPs in the IL-17A and IL-17F genes (rs2275913G>A, rs3748067C>T and rs763780T>C) in the development of gastric cancer, and their interaction with H.pylori infection. A total of 326 patients with gastric cancer and 326 control subjects were consecutively recruited between May 2012 and May 2014. Genotyping of IL-17A rs2275913G>A and rs3748067C>T and IL-17F rs763780T>C was performed in a 384-well plate format on the Sequenom MassARRAY platform. By logistic regression analysis, individuals carrying the GA and AA genotypes of IL-17 rs2275913G>A were significantly associated with an increased risk of gastric cancer when compared with GG genotype, and the adjusted Ors (95% CI) were 1.46 (1.03-2.06) for GA genotype and 2.57 (1.51-4.43) for AA genotype. We observed that the GA+AA genotype of rs2275913 was associated with an increased risk of gastric cancer among *H.pylori* infection subjects (OR=2.21, 95% CI=1.29-3.79). In conclusion, we found that there was a significant association between L-17A rs2275913G>A polymorphism and increased gastric cancer risk, especially in *H.pylori* infection subjects.

Keywords: IL-17, polymorphism, *H.pylori* infection, gastric cancer

Introduction

Gastric cancer (GC) is the fourth most common cancer in the world and the second most common cause of cancer-related death, especially in East Asia [1]. Carcinogenesis of gastric is caused by various risk factors, including genetic predisposition, environment, and microbial infections. Although environmental factors in addition to *Helicobacter pylori* (*H.pylori*) infections have been identified to play important roles in the development of gastric cancer [2-4], only a few patients develop gastric cancer if they were exposed to similar environmental factors. Therefore, some genetic factors may contribute to the development of gastric cancer. Many single-nucleotide polymorphisms (SNPs) have been implicated in gastric carcinogenesis [5, 6].

Chronic inflammation is a well-known risk factor for malignant transformation, but the role inflammation plays in cancer initiation is not well understood [7, 8]. Interleukin-17 (IL-17) is a relative novel cytokine secreted exclusively by activated T-cells that bridges the adaptive and innate immune systems [9, 10]. IL-17A and IL-17F are two important members of the IL-17 cytokine family preferentially produced by helper T 17 (Th17) cells, and they play an important role in the pathogenic activity of the lineage of CD4+ effector cells and multiple proinflammatory mediators [11, 12]. It has been reported that single nucleotide polymorphisms (SNPs) in IL-17A and IL-17F are correlated with the development of gastric cancer [13].

Several studies have indicated that genetic polymorphism in inflammation pathway genes are thought to play a role in the susceptibility of cancer development, including cytokines and their receptors [10, 11]. Increasing evidences have indicated that IL-17A and IL-17F polymorphisms are associated with the risk of a range of tumor, such as gastric cancer, colorectal cancer, oral squamous cell carcinoma, breast cancer and non-small cell lung cancer [14-18]. However, many studies regarding the association between IL-17A and IL-17F gene polymorphisms and susceptibility of gastric cancer are

controversial [14, 19-21]. To further reconcile these conflicting findings and to obtain a more definitive conclusion using multiple genetic statistical models, we conducted a study to investigate the role of three common SNPs in the IL-17A and IL-17F genes (rs2275913G>A, rs3748067C>T and rs763780T>C) in the development of gastric cancer, and their interaction with H.pylori infection.

Materials and methods

Study subjects

A case-control study was taken in our study. A total of 326 patients with gastric cancer and 326 control subjects were consecutively recruited from the Centre Hosptial of Zhumadian between May 2012 and May 2014. All cases were confirmed by histopathological diagnosis. Subjects who received chemotherapy or radiotherapy before surgery were excluded from this study. Gastric cancer patients who had any other malignant tumors and underwent preoperative radiotherapy or chemotherapy were excluded from our study. Control subjects were randomly selected from individuals seeking for routinely heath check-up in the Centre Hospital of Zhumadian during the same period. Control subjects were frequency-matched with cases on age and sex. Control subjects who had a history of cancer were excluded from our study. Blood samples were collected from each participant. Detailed personal information on demographic characteristics, smoking and drinking status were collected by interview.

Data on demographic information were collected using a standard questionnaire by trained staffs, such as sex, age, dietary habits, alcohol consumption, tobacco smoking and family history of cancer.

The *H.pylori* infection of patients and controls was determined using ELISA (Diagnostic Automation, CA, United States) and a rapid urea breath test. Patients with one or both of positive results of the two tests were determined as *H.pylori* infection.

Genotyping

Each included subject was asked to provide 5 ml venous blood for genomic DNA extraction. The blood samples were kept at -20°C until use, with EDTA 0.5 mg/ml used as anticoagulant. Genomic DNA from participants was extracted from EDTA-treated whole blood, using the DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genotyping of IL-17A rs2275913G>A and rs3748067C>T and IL-17F rs763780T>C was performed in a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). The primers for IL-17A rs2275913G>A and rs3748067C>T and IL-17F rs763780T>C were designed by Assay Design 3.1 according to the manufacturer instructions. The primers for IL-17A rs2275913 were 5'-GCAGTTGTGCTCAGCTTCTAA-3' (forward) and 5'-TTCAGGGGTGACACCATTTT-3' (reverse). The primers for IL-17A rs3748067C>T were 5'-CTGTTTCCATGGCTGCAGGTC-3' (forward) and 5'-TGGTGAGCTGGTTCTGCACTT-3' (reverse). The primers for IL-17F rs763780 were 5'-CTGTTTCCATGGGTGCACCTC-3' (forward) and 5'-TCCTGACTGTTCTCCGCACCT-3' (reverse). Briefly PCR was performed in a final volume of 5 μL reaction solution with 50 ng genomic DNA template using GeneAmp® PCR System 9700 with Dual 384-Well Sample Block Module (Applied Biosystems, Carlsbad, USA). The results were confirmed by sequencing the PCR products using an automated sequencing system. Reproducibility was verified by repeat analysis of a randomly chosen subgroup of 5% of the subjects, and results of all duplicated samples were 100% consistent.

Statistical analysis

Continuous variables were shown as the mean and standard deviation (SD), and categorical variables were shown as frequencies and percentage (%). A chi-square (χ^2) test was taken to compare the distributions in sex, age, dietary habits, alcohol consumption, tobacco smoking and family history of cancer as well as genotype and alleles distributions between case and control groups. χ^2 test was taken to evaluate the Hardy-Weinberg equilibriums (HWE) in control groups. Unconditional logistic regression analysis was used to examine the association between the three SNPs and risk of gastric cancer, and ORs and 95% CIs were used to calculate the relative risk for each SNP. The ORs (95% CI) were adjusted for potential confounding factors. Homozygotes of the most frequent genotype were used as the reference group. All

Table 1. Demographic and clinical characteristics of gastric cancer patients and control subjects

	Patients N=326	%	Controls N=326	%	*t* or χ^2	*P* value
Age, years						
<60	183	56.13	178	54.60		
≥60	143	43.87	148	45.40	0.15	0.69
Sex						
Female	116	35.58	116	35.58		
Male	210	64.42	210	64.42	0.00	1.00
Cancer history in the first relatives						
No	302	92.64	323	99.08		
Yes	24	7.36	3	0.92	17.04	<0.001
Alcohol drinking, n (%)						
Never	181	55.52	207	63.50		
Current or ever	145	44.48	119	36.50	4.30	0.04
Cigarette smokers, n (%)						
Never	214	65.64	227	69.63		
Current or ever	112	34.36	99	30.37	1.18	0.28
H.pylori infection						
Negative	138	42.33	232	71.17		
Positive	188	57.67	94	28.83	55.21	<0.001

Table 2. Genotype and allele frequencies of IL-17A rs2275913G>A and rs3748067C>T and IL-17F rs763780T>C in gastric cancer patients and control subjects

Genotype of IL-17	Patients	%	Controls	%	χ^2	*P* value	Hardy-Weinberg Equilibrium	Minor allele frequency (MAF)	
								In controls	In database
rs2275913G>A									
GG	121	37.12	161	49.39					
GA	149	45.70	136	41.72					
AA	56	17.18	29	8.89	14.84	0.001	0.97	0.3075	0.2927
rs3748067C>T									
TT	274	84.05	286	87.73					
TC	34	10.43	30	9.20					
CC	18	5.52	10	3.07	2.79	0.25	<0.001	0.0767	0.0769
rs763780T>C									
CC	266	81.59	278	85.28					
CT	38	11.66	33	10.12					
TT	22	6.75	15	4.60	1.94	0.38	<0.001	0.0966	0.0935

P-values were two-sided, and a *P*-value < 0.05 was considered statistically significant. All of these statistical tests were done using Stata (version 9.0; StataCorp, College Station, TX) software programs.

Results

A total of 326 gastric cancer patients (116 females and 201 males) and 326 control subjects (116 females and 201 males) were included in our study (**Table 1**). The mean age was 56.50±10.52 years old for gastric cancer patients, and the mean age was 57.40±10.75 years old for control subjects. There were no statistically significant differences between cases and controls in terms of sex and age (*P*>0.05). We found that gastric cancer patients were more likely to be alcohol drinkers and cigarette smokers, have cancer history in the first relatives, and be infected with *H.pylori* (*P*<0.05).

Table 3. Association of IL-17A rs2275913G>A and rs3748067C>T and IL-17F rs763780T>C with risk of gastric cancer

Genotype of IL-17	Patients	%	Controls	%	OR (95% CI)[1]	*P* value
rs2275913G>A						
GG	121	37.12	161	49.39	Ref.	
GA	149	45.7	136	41.72	1.46 (1.03-2.06)	0.03
AA	56	17.18	29	8.89	2.57 (1.51-4.43)	<0.001
GA+AA	205	62.88	165	50.61	1.65 (1.19-2.29)	0.002
rs3748067C>T						
TT	274	84.05	286	87.73	Ref.	
TC	34	10.43	30	9.2	1.18 (0.68-2.06)	0.52
CC	18	5.52	10	3.07	1.88 (0.80-4.64)	0.11
TC+CC	52	15.95	40	12.27	1.36 (0.85-2.17)	0.18
rs763780T>C						
CC	266	81.59	278	85.28	Ref.	
CT	38	11.66	33	10.12	1.20 (0.71-2.04)	0.46
TT	22	6.75	15	4.6	1.53 (0.74-3.25)	0.21
CT+TT	60	18.41	48	14.72	1.31 (0.85-2.03)	0.21

[1]Adjusted for sex, age, cancer history in the first relatives, alcohol drinking, cigarette smoking and *H.pylori* infection.

Table 4. Association between IL-17A rs2275913G>A gene polymorphism and gastric cancer risk Stratified by *Helicobacter pylori* infection

Helicobacter pylori	GG genotype of rs2275913		GA+AA genotype of rs2275913		OR (95% CI)[1]	*P* value
	Patients	Controls	Patients	Controls		
Negative	59	112	79	120	1.25 (0.80-1.96)	0.20
Positive	62	49	126	45	2.21 (1.29-3.79)	0.002

[1]Adjusted for sex, age, cancer history in the first relatives, alcohol drinking and cigarette smoking.

There were significant differences in IL-17A rs2275913G>A genotype distributions between patients and controls (**Table 2**). The frequencies of GA and AA genotype frequencies were significantly higher in cases than those in controls (*P*=0.001). Moreover, the genotype distributions of IL-17A rs2275913G>A were in HWE in controls, while genotype distributions of IL-17A rs3748067C>T and IL-17F rs763780T>C were not. The minor allele frequency (MAF) of IL-17A rs2275913G>A, rs3748067C>T and IL-17F rs763780T>C were similar to the MAF in the dbSNP database.

By logistic regression analysis, individuals carrying the GA and AA genotypes of IL-17 rs2275913G>A were significantly associated with an increased risk of gastric cancer when compared with the GG genotype, and the adjusted Ors (95% CI) were 1.46 (1.03-2.06) for the GA genotype and 2.57 (1.51-4.43) for the AA genotype (**Table 3**). Moreover, the GA+AA genotype had a significantly increased risk for gastric cancer (OR=1.65, 95% CI=1.19-2.29). However, no significant positive association was observed with the risk of gastric cancer in the association analysis of the IL-17A rs3748067C>T and IL-17F rs763780T>C polymorphisms.

By stratified analysis, we observed that the GA+AA genotype of rs2275913 was associated with an increased risk of gastric cancer among *H.pylori* infection subjects (**Table 4**), and the adjusted OR (95% CI) for the GA+AA genotype was 2.21 (1.29-3.79).

Discussion

It is well known that genetic susceptibility to cancers have obtained an increasing attention to investigate the polymorphisms of genes involved in tumourigenesis. Inflammation and related cytokines have a critical role in the epithelium transformation from ulcer to gastric cancer. The inflammatory state is a essential

step to maintain and promote cancer progression and accomplish the full malignant phenotype, such as tumor tissue rebuilding, angiogenesis, metastasis and suppress the innate anticancer immune response [22]. Moreover, genetic variations and epigenetic mutation may trigger cell transformation and maintains the autonomous proliferation of the transformed cells to cancer proliferation.

IL-17 is a critical inflammatory cytokine to connect the innate and adaptive immunity [23]. It has been reported that IL-17 is a necessary proinflammatory cytokine to evoke many cytokines and chemokines secretion through various cell types [24]. IL-17 could also promote the expression of antimicrobial peptides and facilitates host defense against infections [25, 26]. Our study reported that IL-17A rs2275913G>A polymorphism is associated with susceptibility of gastric cancer. The IL-17A rs2275913G>A is located in the 5' region near the IL-17A gene, and it may regulate the gene transcription. Therefore, the IL-17A rs2275913G>A polymorphism many influence the risk of gastric cancer.

Recently, many epidemiological studies have investigated the role of IL-17 gene polymorphisms in the susceptibility of gastric cancer, but the results are conflicting with these studies [10, 13, 14, 19, 21, 27-29]. Shibata et al. firstly reported the association between IL-17A and IL-17F gene polymorphism and gastric carcinogenesis, and they reported that AA genotype of IL-17A rs2275913G>A was associated with an increased risk of gastric cancer, especially for intestinal-type cancer [10]. Wu et al. conducted a study in a Chinese population, and they did not find that IL-17A rs2275913G>A could influence the susceptibility to gastric cancer [13]. Arisawa et al. reported that AA genotype of IL-17A rs2275913G>A was associated with an increased risk of gastric cancer [19]. Rafiei et al. conducted a study in an Iranian population, and they suggested that A allele of IL-17A rs2275913G>A significantly increased gastric cancer risk [21]. Other three studies in Chinese populations reported a positive association between IL-17A rs2275913G>A gene polymorphism and susceptibility to gastric cancer [14, 20, 28]. However, Gao et al. reported that no association was identified between IL-17A rs2275913G>A gene polymorphism and development of gastric cancer [29]. The discrepancy may be attributed to different sample size, gastric cancer selection and ethnicities of above-mentioned studies.

We also found that the role of IL-17A rs2275913G>A polymorphism with susceptibility to gastric cancer was modified by *H.pylori* infection. Previous studies reported that Interleukin-17 (IL-17) is an important player in the pathophysiology of infectious and immune-mediated gastrointestinal diseases, and H. pylori infection increases IL-17 in the gastric mucosa of humans [30]. However, the underlying mechanism involved in the association between IL-17A rs2275913G>A polymorphism and *H.pylori* infection is not well understood. Therefore, further studies are greatly needed to confirm their association.

Three limitations should be considered in this study. First, IL-17A rs3748067C>T and IL-17F rs763780T>C did not confirm with Hardy–Weinberg equilibrium, which suggests that the selected patients and controls could not be representative of the general population. Second, other genetic polymorphisms of cytokine genes may influence the development of gastric cancer in addition to IL-17 genes. Third, the present sample size was relatively small, although the number of study participants met the requirement for analysis. Therefore, larger sample studies should be conducted in the future.

In conclusion, we found that there was a significant association between L-17A rs2275913G>A polymorphism and increased gastric cancer risk, especially in *H.pylori* infection subjects. Further well designed and large sample size studies are greatly needed to confirm our results.

Disclosure of conflict of interest

None.

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