# Association between *IL-17A* and *IL-17F* gene polymorphisms and risk of gastric cancer in a Chinese population

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**ABSTRACT.** We conducted a case-control study to investigate the role of interleukin-17A (*IL-17A*) rs2275913 G > A and *IL-17F* rs763780 T > C polymorphisms in the development of gastric cancer. A hospital-based case-control design was performed, and 153 patients and 207 control subjects were consecutively selected from the Third Affiliated Hospital between May 2013 and December 2014. Polymerase chain reaction-restriction fragment length polymorphism was used to genotype for *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C. The genotypes of *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C did not deviate from Hardy-Weinberg equilibrium (P values were 0.44 and 0.11, respectively). By unconditional logistic regression analysis, we observed that the GG genotype of rs2275913 was associated with an increased risk of gastric cancer compared to the AA genotype [odds

ratio (OR) = 2.66; 95% confidence interval (CI) = 1.26-5.66]. The AG + GG genotype of rs2275913 increased the susceptibility to gastric cancer compared to the AA genotype, and the adjusted OR (95%CI) was 2.66 (1.26-5.66). Moreover, the GG genotype of rs2275913 was correlated with an elevated risk of gastric cancer when compared with the AA + AG genotype (OR = 2.15; 95%CI = 1.08-4.34). In conclusion, we found that the *IL-17A* rs2275913 G > A gene polymorphism was significantly associated with an increased risk of gastric cancer in co-dominant, dominant, and recessive models.

**Key words:** *IL-17A* rs2275913 G > A; *IL-17F* rs763780 T > C; Polymorphisms; Gastric cancer

## INTRODUCTION

Gastric cancer is one of the most common malignant tumors in both mortality and morbidity worldwide, especially in developing countries (International Agency for Research on Cancer, 2012). It is well known that infection with *Helicobacter pylori* plays an important role in the development of gastric cancer, and *H. pylori* has been categorized as a class I carcinogen that infects the stomach of about half of the global population (Alberts et al., 2003; Mantovani et al., 2008). Moreover, previous studies have reported that many environmental and lifestyle factors could contribute to the risk of developing gastric cancer, such as consumption of preserved food containing carcinogenic nitrates, tobacco smoking, alcohol consumption, and overweight (Alberts et al., 2003). In additional, previous studies have reported that some genetic factors may contribute to the risk of developing gastric cancer, such as miRNA-196a-2, stromelysin-1 promoter gene, DNA repair and xenobiotic pathway gene, TP53 condon 72 gene and cytotoxic T-lymphocyte antigen 4 gene (Krishnaveni et al., 2015; Tang et al., 2015; Zha et al., 2015; Ghosh et al., 2016; Song et al., 2016).

Chronic inflammation is a well-known risk factor for malignant transformation, but the role of inflammation in carcinogenesis is not very clear or understood. *IL-17A* and *IL-17F* are two important members of the IL-17 family, and the two genes are both located at chromosome 6q12 and are composed of three exons and two introns. Previous studies have reported the association between *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C single nucleotide polymorphisms (SNPs) and gastric cancer risk, but the results are inconclusive (Rafiei et al., 2013; Qinghai et al., 2014; Gao et al., 2015; Hou and Yang, 2015; Qi et al., 2015). We carried out a case-control study to further examine the role of *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C polymorphisms in the risk of developing gastric cancer.

## MATERIAL AND METHODS

### Patients

A hospital-based case-control design was taken in this study, and 153 patients were consecutively selected from the Department of Gastrointestinal Surgery of the Third Affiliated Hospital between May 2013 and December 2014. All the gastric cancer patients

were independently identified by two pathologists. The exclusion criteria were patients who had other malignant cancers, recurrent cancer, or renal or liver disease. During the same time period, 207 healthy subjects were randomly selected from individuals who underwent a regular health check-up in the Third Affiliated Hospital. All the control subjects were free of cancer and digestive system diseases.

Detailed environmental and clinical data were collected from the medical records of all subjects, including age, sex, cancer history in the first relatives, alcohol drinking, tobacco smoking, tumor-node-metastasis (TNM) stage at diagnosis. Blood samples (5 mL) and signed informed consent forms were collected from all patients and controls before their participation in the study. The protocol of this project was approved by the Ethics Committee of the Third Affiliated Hospital.

## DNA extraction and SNP genotyping

The collected blood samples were kept in ethylene diamine tetra-acetic acid (EDTA)-coated tubes and stored at -20°C until use. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to genotype for *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C. The primers for PCR were designed using Sequenom Assay Design 3.1 software (San Diego, CA, USA), and primers for PCR and their specific restriction enzyme were described in Table 1. The amplification reactions began with an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 62°C for 60 s, and extension at 72°C for 60 s, with a final extension at 72°C for 10 min. Digestion products were verified by electrophoresis on ethidium bromide-stained agarose gels.

**Table 1.** Primers, product size, and restriction enzymes used for *IL-17A*rs2275913G>A and *IL-17F* rs763780.

| *IL-17* gene | PCR primer sequence (5'→3') | Restriction enzymes |
|---|---|---|
| rs2275913G>A | 5'-GCCCTTCCCATTTTCCTTCAGA-3' | *Eco*NI |
| | 5'-CCAATCAACTGGGGATGGATGA-3' | |
| rs763780T>C | 5'-CTGTTTCCATCCGTGCAGGTC-3' | *Nla*III |
| | 5'-TGGTGACTGTTGGCTGCACCT-3' | |

## Statistical analysis

The chi-square test was used to analyze whether the genotype distributions of *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C deviated from the Hardy-Weinberg equilibrium (HWE). The association between *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C gene polymorphisms and gastric cancer risk was described by the odds ratio (OR) and 95% confidence interval (95%CI). Co-dominant, dominant, and recessive models were used to assess the association between *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C gene polymorphisms and development of gastric cancer. The main homozygous genotype of *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C was used as a reference group for analysis. SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. A P value < 0.05 was considered as statistically significant.

## RESULTS

By chi-square test, no statistically significant differences were found between gastric

cancer patients and control subjects in terms of age ($\chi^2 = 0.13$, P = 0.72) and tobacco smoking ($\chi^2 = 3.17$, P = 0.08) (Table 2). However, we found that patients with gastric cancer were more likely to be males ($\chi^2 = 5.41$, P = 0.02), have cancer history in the first relatives ($\chi^2 = 5.52$, P = 0.02), and have a habit of alcohol consumption ($\chi^2 = 14.71$, P < 0.001) (Table 2). Of the 153 patients with gastric cancer, 62 (40.52%) patients were at TNM stage I-II, 91 (59.48%) were at TNM stage III-IV.

**Table 2.** Lifestyle and clinical characteristics of patients with gastric cancer and control subjects.

| Variables | Patients (N = 153) | % | Controls (N = 207) | % | $\chi^2$-test | P value |
|---|---|---|---|---|---|---|
| Age, years | | | | | | |
| <50 | 68 | 44.44 | 88 | 42.51 | | - |
| ≥50 | 85 | 55.56 | 119 | 57.49 | 0.13 | 0.72 |
| Gender | | | | | | |
| Female | 46 | 30.07 | 87 | 42.03 | | - |
| Male | 107 | 69.93 | 120 | 57.97 | 5.41 | 0.02 |
| Cancer history in the first relatives | | | | | | |
| No | 140 | 91.50 | 201 | 97.10 | | - |
| Yes | 13 | 8.50 | 6 | 2.90 | 5.52 | 0.02 |
| Alcohol consumption | | | | | | |
| No | 68 | 44.44 | 134 | 64.73 | | - |
| Yes | 85 | 55.56 | 73 | 35.27 | 14.71 | <0.001 |
| Tobacco smoking | | | | | | |
| No | 84 | 54.90 | 94 | 45.41 | | - |
| Yes | 69 | 45.10 | 113 | 54.59 | 3.17 | 0.08 |
| TNM stage at diagnosis | | | | | | |
| I-II | 62 | 40.52 | | | | |
| III-IV | 91 | 59.48 | | | | |

Genotype distributions of *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C in gastric cancer patients and control subjects are described in Table 3. By chi-square test, a significant difference was found in genotype distributions of *IL-17A* rs2275913 G > A, while no significant difference was found in *IL-17F* rs763780 T > C genotypes. The genotypes of *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C did not deviated from HWE (P values were 0.44 and 0.11, respectively). Furthermore, the minor allele frequencies of *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C in controls were similar with those from the dbSNP database (http://www.ncbi.nlm.nih.gov/snp).

**Table 3**. Genotype distributions of *IL-17A*rs2275913G>A and *IL-17F* rs763780T>C in gastric cancer patients and control subjects.

| *IL-17* | Patients (N = 153) | % | Controls (N = 207) | % | P for HWE In controls | $\chi^2$ test | P value | Minor allele frequency | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | In database | In controls |
| rs2275913 | | | | | | | | | |
| GG | 51 | 33.33 | 95 | 45.89 | | | | | |
| GA | 76 | 49.67 | 94 | 45.41 | | | | | |
| AA | 26 | 16.99 | 18 | 8.70 | 0.44 | 8.72 | 0.01 | 0.2927 | 0.3140 |
| rs763780 | | | | | | | | | |
| TT | 114 | 74.51 | 165 | 79.71 | | | | | |
| TC | 29 | 18.95 | 37 | 17.87 | | | | | |
| CC | 10 | 6.54 | 5 | 2.42 | 0.11 | 3.94 | 0.14 | 0.0935 | 0.1135 |

By unconditional logistic regression analysis, we observed that the GG genotype of rs2275913 was associated with an increased risk of gastric cancer compared to the AA

genotype (OR = 2.66; 95%CI = 1.26-5.66) (Table 4). The AG + GG genotype of rs2275913 significantly increased the susceptibility to gastric cancer compared to the AA genotype, and the adjusted OR (95%CI) was 2.66 (1.26-5.66). Moreover, the GG genotype of rs2275913 was correlated with an elevated risk of gastric cancer when compared with the AA + AG genotype (OR = 2.15; 95%CI = 1.08-4.34). However, no significant association was found between the *IL-17F* rs763780 T > C gene polymorphism and development of gastric cancer in co-dominant, dominant, and recessive models.

**Table 4.** Association between *IL-17A*rs2275913G>A and *IL-17F* rs763780T>C gene polymorphisms and gastric cancer risk.

| *IL-17* | Patients (N = 153) | % | Controls (N = 207) | % | OR (95%CI)[1] | P value |
|---|---|---|---|---|---|---|
| rs2275913 | | | | | | |
| Co-dominant | | | | | | |
| AA | 51 | 33.33 | 95 | 45.89 | 1.0 (Ref.) | - |
| AG | 76 | 49.67 | 94 | 45.41 | 1.51 (0.93-2.44) | 0.08 |
| GG | 26 | 16.99 | 18 | 8.70 | 2.66 (1.26-5.66) | 0.005 |
| Dominant | | | | | | |
| AA | 51 | 33.33 | 95 | 45.89 | 1.0 (Ref.) | - |
| AG+GG | 102 | 66.67 | 112 | 54.11 | 1.70 (1.08-2.68) | 0.02 |
| Recessive | | | | | | |
| AA+AG | 127 | 83.01 | 189 | 91.30 | 1.0 (Ref.) | - |
| GG | 26 | 16.99 | 18 | 8.70 | 2.15 (1.08-4.34) | 0.02 |
| rs763780 | | | | | | |
| Co-dominant | | | | | | |
| CC | 114 | 74.51 | 165 | 79.71 | 1.0 (Ref.) | - |
| CT | 29 | 18.95 | 37 | 17.87 | 1.13 (0.63-2.02) | 0.65 |
| TT | 10 | 6.54 | 5 | 2.42 | 2.89 (0.87-11.05) | 0.07 |
| Dominant | | | | | | |
| CC | 114 | 74.51 | 165 | 79.71 | 1.0 (Ref.) | - |
| CT+TT | 39 | 25.49 | 42 | 20.29 | 1.34 (0.79-2.28) | 0.24 |
| Recessive | | | | | | |
| CC+CT | 143 | 93.46 | 202 | 97.58 | 1.0 (Ref.) | - |
| TT | 10 | 6.54 | 5 | 2.42 | 2.83 (0.86-10.74) | 0.06 |

[1]Adjusted for gender, age, family history of cancer and alcohol drinking.

## DISCUSSION

We conducted a case-control study to investigate the association between *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C gene polymorphisms and the risk of gastric cancer in a Chinese population. Our results suggested that the *IL-17A* rs2275913 G > A gene polymorphism is significantly associated with an increased risk of gastric cancer.

To date, many molecular epidemiological studies have been conducted to evaluate the relationship between *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C polymorphisms and several kinds of cancers and diseases, such as acute myeloid leukemia, non-small cell lung cancer, colorectal cancer, papillary thyroid cancer, cervical cancer, hepatocellular carcinoma, and coronary artery disease (Wróbel et al., 2014; Cheng et al., 2015; ELBassuoni et al., 2015; Geng et al., 2015; Ma et al., 2015; Lee et al., 2015; Lv et al., 2015).

For the correlation between *IL-17* gene polymorphisms and development of gastric cancer, several previous studies have reported their association (Rafiei et al., 2013; Qinghai et al., 2014; Wang et al., 2014; Zhang et al., 2014; Gao et al., 2015; Hou and Yang, 2015; Qi et al., 2015), but they also reported inconclusive results. Zhang et al. (2014) conducted a study in a Chinese population and found that the rs2275913 G > A and rs763780 T > C polymorphisms

contributed to the development of gastric cancer. Qinghai et al. (2014) assessed the correlation between the six common SNPs in *IL-17* and gastric cancer risk and found that rs2275913, rs3748067, and rs9382084 increased the development of gastric cancer. Wang et al. (2014) conducted a case-control study in a Chinese population and suggested that rs2275913 and rs3748067 polymorphisms were correlated with an increased risk in a Chinese population. Gao et al. (2015) conducted a study in a Chinese population and reported rs763780 T > C was correlated with risk of gastric cancer, but also reported that no association was identified between rs2275913 and rs3748067 polymorphisms and the risk of gastric cancer. Our study reported some similar results with the previous studies. In our study, we examined 153 gastric cancer patients and 207 control subjects, and found that the rs2275913 G > A gene polymorphism could influence the development of gastric cancer (Qinghai et al., 2014; Wang et al., 2014; Zhang et al., 2014; Gao et al., 2015). However, we did not observe any significant association between the rs763780 T > C genetic polymorphism and gastric cancer risk, which is inconsistent with previous results (Zhang et al., 2014; Gao et al., 2015). The discrepancies of the above mentioned studies may be explained by different study populations, source of patients and controls, and sample sizes.

Two limitations should be considered in this study. First, the hospital-based case-control study may cause selective bias. However, the genotype distributions of *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C did not deviate from HWE, which suggests that our study subjects could represent the general population. Second, the sample size of the included subjects is small and this may cause low power in the determination of statistical differences between groups. Further large sample studies are greatly needed to confirm our study.

In conclusion, we found that the *IL-17A* rs2275913 G > A gene polymorphism was significantly associated with an increased risk of gastric cancer in co-dominant, dominant, and recessive models. Future studies using larger sample sizes should be employed to assess the role of *IL-17A* rs2275913 G > A and *IL-17F* rs763780 T > C polymorphisms in risk of gastric cancer.

## Conflicts of interest

The authors declare no conflict of interest.

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