RESEARCH ARTICLE

Meta-analysis of Associations between Interleukin-17 Gene Polymorphisms and Risk of Gastric Cancer

Hui Yu[1]*, Si Sun[1], Fang Liu[2], Qing-Hua Xu[2]

Abstract

Background: Previous studies have indicated that single nucleotide polymorphisms (SNPs) of the interleukin-17 (IL-17) gene are associated with an increased risk of gastric cancer. However, the findings were inconsistent. Materials and Methods: To provide a more reliable estimation of the association between SNPs in the IL-17 gene and the susceptibility to gastric cancer, we searched PubMed, CNKI, and Wan Fang databases and selected finally six studies covering 2,366 cases and 3,205 controls to perform a meta-analysis. Results: Statistical analyses showed that an rs2275913 polymorphism within the IL-17A gene was significantly associated with an increased risk of gastric cancer using a generalized odds ratio (ORG, a model-free approach). Moreover, we also found that the 'A' allele carriers of IL-17A rs2275913 had a significant link with clinicopathological features. However, no significant positive signals were observed in the association analysis of the rs3748067 and rs763780 polymorphisms with the risk of gastric cancer in IL-17A and IL-17F, respectively. Conclusions: Despite some limitations, the present meta-analysis provided a more precise estimation of the relationship between the IL-17 gene SNPs and gastric cancer risk compared with individual studies.

Keywords: Interleukin-17 - gastric cancer - single nucleotide polymorphism - meta-analysis

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Introduction

Gastric cancer is well-documented as one of the most common cancers linked to a high incidence of fatality worldwide (Hartgrink et al., 2009; Siegel et al., 2012). Although genetic and environmental factors in addition to *Helicobacter pylori* infections have been identified to play important roles in the development of gastric cancer (Houghton and Wang, 2005; Merchant, 2005; Dong et al., 2008), the precise etiology of the disease remains unclear. Notably, more and more studies have indicated that genetic variants of interleukin (IL) family genes may be involved in the development of gastric cancer (El-Omar et al., 2000; Xue et al., 2012; Yang et al., 2013; Pan et al., 2014; Zhu et al., 2014).

Interleukin-17 (IL-17) is a cytokine secreted exclusively by activated T-cells that bridges the adaptive and innate immune systems (Moseley et al., 2003; Shibata et al., 2009). IL-17A and IL-17F are important members of the IL-17 cytokine family preferentially produced by helper T 17 (Th17) cells, which are responsible for the pathogenic activity of the lineage of CD4+ effector cells and multiple proinflammatory mediators (Rutitzky et al., 2005; Ishigame et al., 2009). A previous study has reported that IL-17A and IL-17F single nucleotide polymorphisms (SNPs) are associated with the risk of gastric cancer (Wu et al., 2010). However, subsequent replication studies for the association between IL-17A and IL-17F variants and gastric cancer risk are controversial (Shibata et al., 2009; Arisawa et al., 2012; Rafiei et al., 2013; Zhang et al., 2014; Zhu et al., 2014). The discrepancy may be attributed to the relatively small sample size of previous studies and the genetic heterogeneity of polymorphisms in gastric cancer among different populations.

To further reconcile these conflicting findings and to obtain a more definitive conclusion using multiple genetic statistical models, a meta-analysis using three SNPs within the IL-17A and IL-17F genes was conducted to collectively analyze existing comparative studies.

Materials and Methods

Literature search

The studies included in the meta-analysis were selected by searching the PubMed, CNKI (http://www.cnki.net), and Wan Fang (http://www.wanfangdata.com.cn) databases through Feb 2014 using the following keywords and subject terms: "Interleukin-17 gene or IL-17" AND "gastric cancer or gastric carcinoma". All references in these studies were examined to identify additional research not indexed by the databases. We selected only published articles written in English or in Chinese.

*[1]Department of Medical Oncology, Fudan University Shanghai Cancer Center; Department of Oncology, Shanghai Medical College, Fudan University, [2]Fudan University Shanghai Cancer Center-Institute Mérieux Laboratory, Shanghai, China *For correspondence: yhui30@hotmail.com*

Inclusion criteria

Studies included in the current meta-analysis had to meet the following criteria: (1) a case-control study design; (2) a focus on the association of IL-17 SNPs with gastric cancer risk and publication in a peer-reviewed journal; (3) sufficient data to calculate the odds ratio (OR), confidence interval (CI) and *p* value; (4) specified genotyping methods (including through provision of an appropriate reference); and (5) genotype distribution of all control groups in Hardy-Weinberg equilibrium (HWE).

Data extraction

Data were extracted from eligible publications independently by two of the authors (Hui Yu and Si Sun.) with any disagreement resolved by discussion between them. The following data were collected from each study: first author's name, publication year, sample size, country, ethnicity, age, genotyping method, and SNPs studied.

Quality assessment

The quality of the studies was independently assessed using a set of predetermined criteria that was extracted and modified from previous study (Li et al., 2013) (Table 1). The articles with higher scores (≥12) presented "high quality". The scores of all include articles in this meta-analysis were shown in Table 2.

Statistical analyses

All analyses were calculated using Stata software (V12.0, Stata, College Station, TX, USA) and the RevMan (v.5.1) program (http://www.cochrane.org/revman). To evaluate the association between IL-17 SNPs and gastric cancer, the significance of the pooled odds ratios (OR) were determined by a Z test. The Q-test and I2 statistic were used to investigate the degree of heterogeneity between the studies. Either a random-effect model or a fixed-effect model was chosen to calculate the subtotal OR and 95% CI (for the pooled odds ratio) based upon whether there was significant difference in heterogeneity among these studies. If the Q-test resulted in a *p* value >0.05, indicating a lack of heterogeneity, the pooled OR estimate of each study was calculated using a fixed-effect model (the Mantel-Haenszel method). If the Q-test resulted in a *p* value<0.05, the pooled OR estimate of each study was calculated with a random-effect model (the DerSimonian

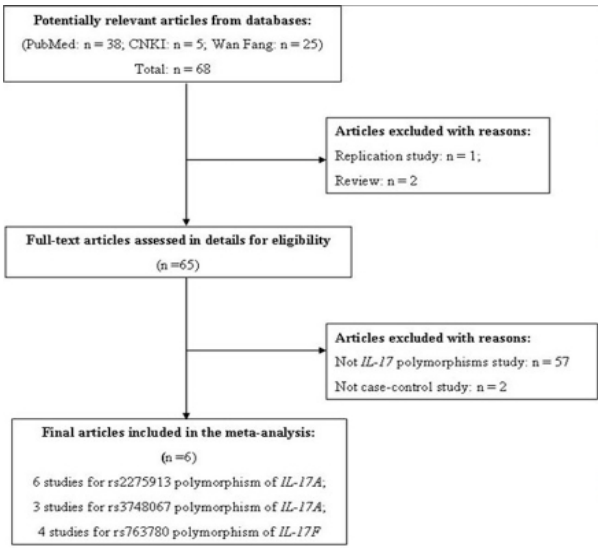

Figure 1. Flow Diagram of the Publication Selection Process

Table 1. Scale for Quality Assessment

Criterion	Score
Source of cases	
Selected from population or cancer registry	3
Selected from hospital	2
Selected from pathology archives, but without description	1
Not described	0
Source of controls	
Population-based	3
Blood donors or volunteers	2
Hospital-based (caner-free patients)	1
Not described	0
Case-control match	
Matched by age and gender	3
Not matched by age and gender	0
Specimens used for determining genotypes	
White blood cells or normal tissues	3
Tumor tissue or exfoliated cells of tissue	0
Hardy-Weinberg equilibrium in controls	
Hardy-Weinberg equilibrium	3
Hardy-Weinberg disequilibrium	0
Total sample size	
>1000	3
>500 and <1000	2
>200 and <500	1
<200	0

Table 2. Characteristics of Case-control Studies of between IL-17 SNPs and Gastric Cancer Included in the Meta-analysis

Source	Subject	n	Country	Ethnicity	Age (mean±SD)	Genotyping	SNPs studied	Quality score
Shibata et al. 2009	Cases	287	Japan	Japanese	65.0±11.8	PCR-SSCP	rs2275913, rs763780	14
	Controls	524			55.7±18.3			
Wu et al. 2010	Cases	1010	China	Chinese	56.7±12.6	PCR-RFLP	rs2275913, rs763780	17
	Controls	800			55.9±14.9			
Arisawa et al. 2012	Cases	337	Japan	Japanese	65.3±11.4	PCR-SSCP	rs2275913, rs3748067	14
	Controls	587			61.4±13.7			
Rafiei et al. 2013	Cases	161	Iran	Iranians	62.6±12.4	PCR-RFLP	rs2275913	15
	Controls	171			60.8±12.8			
Zhang et al. 2013	Cases	260	China	Chinese	60.6±10.7	Sequenom-MassARRAY	rs2275913, rs3748067, rs763780	16
	Controls	512			51.3±11.2			
Zhu et al. 2014	Cases	311	China	Chinese	57.5±11.3	Sequenom-MassARRAY	rs2275913, rs3748067, rs3819025, rs763780, rs9382084, rs12203582	14
	Controls	611			56.7±12.7			

*SD, standard deviation; PCR-SSCP, polymerase chain reaction-single strand conformation polymorphism; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism

and Laird method). In addition to the pooled OR of the various genetic models, a generalized odds ratio (ORG) was calculated in this analysis by the "ORGGASMA" software (available at http://biomath.med.uth.gr), which is model-free approach and utilizes the complete genotype distribution and provides an estimate of the overall magnitude of the association (Zintzaras, 2010).

To assess the stability of results, sensitivity analysis was performed using a stepwise process in the meta-analysis, namely, a single study in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled OR. Publication bias was examined by a Begg's and Egger's tests (p<0.05 was considered representative of statistically significant publication bias) (Begg and Mazumdar, 1994; Egger et al., 1997).

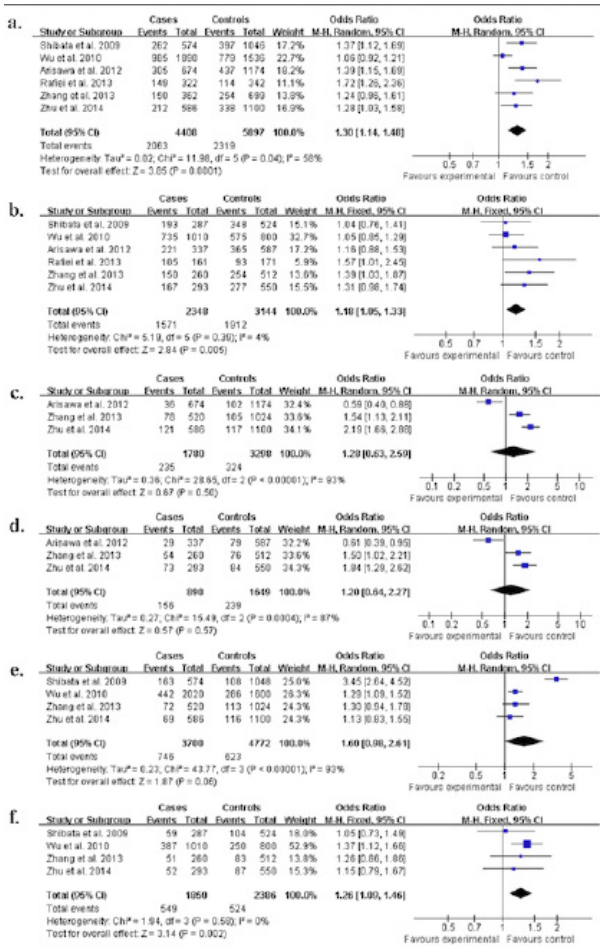

Figure 2. Forest plot of the association between the IL-17 gene SNPs and gastric cancer. a: for rs2275913, A *vs* G; **b:** for rs2275913, AA+AG *vs* GG; **c:** for 3748067, T vs. C; **d:** for 3748067, TT+TC *vs* CC; **e**: for 763780, C *vs* T; **f:** for 763780, CC+CT *vs* TT

Results

Results of literature search

A total of sixty-eight articles were identified in the initial search (Figure 1). Three of these studies were excluded because they were replicates (n=1) and review articles (n=2). In addition, sixty-five of the remaining studies were excluded after reviewing of the abstracts because they were not IL-17 polymorphism studies (n=57) and not case-control studies (n=2). Finally, six articles were selected for inclusion in the current meta-analysis. The main characteristics of these six studies are presented in Table 2.

Meta-analysis

In the present study, to obtain more conclusive results, we evaluated whether the SNPs within the IL-17 gene were associated with gastric cancer risk through multiple genetic models combined with a model-free approach, ORG. The results of the statistical analyses showed that rs2275913 polymorphism within the IL-17A was significantly associated with an increased risk of gastric cancer in multiple genetic comparison models (for rs2275913, A *vs* G: OR=1.30, 95% CI=1.14-1.48, p=0.0001, random-effect model; AA *vs* GG, OR=1.87, 95% CI=1.36-2.56, p=0.0001, random-effect model; AA+AG *vs* GG, OR=1.18, 95% CI=1.05-1.33, p=0.005, fixed-effect model; ORG=1.37, 95% CI=1.13-1.67, fixed-effect model). However, no significant positive signals were observed with the risk of gastric cancer in the association analysis of the rs3748067 and rs763780 polymorphisms in the IL-17A and IL-17F, respectively (Table 3 and Figure 2).

In addition, an association analysis of the 'A' allele in carriers of the IL-17A SNP rs2275913 with clinicopathological features was performed in the current study. We found that 'A' allele carriers of IL-17A rs2275913 had a significantly increased risk for noncardia (OR=1.37, 95% CI=1.16-1.62, p=0.0003, random-effect model), positive *helicobacter pylori* infection (OR=1.61, 95% CI = 1.28-2.02, p=0.0001, fixed-effect model), well differentiated gastric cancer (OR=2.42, 95% CI=1.18-4.97, p=0.02, random-effect model) and TNM I/II stage (OR=1.66, 95% CI=1.17-2.34, p=0.004, random-effect model), compared with GG genotype carriers (Table 4).

Sensitivity analysis and publication bias

By using a stepwise process, meta-analysis was performed repeatedly when each particular study was sequentially excluded. For rs2275913, the removal of

Table 3. Summary Odds Ratio (OR) and 95% Confidence Intervals (CI) of IL-17 SNPs and Gastric Cancer

SNP	Study included	OR (95% CI)	$p^{\#}$	OR (95% CI)	$p^{\#}$	OR (95% CI)	$p^{\#}$	ORG[§] OR (95% CI)	$p^{\ddagger}$
rs2275913	6	A *vs* G 1.30 (1.14-1.48)*	0.0001	AA *vs* GG 1.87 (1.36-2.56)*	0.0001	(AA+AG) *vs* GG 1.18 (1.05-1.33)†	0.005	1.37 (1.13-1.67)†	0.8
rs3748067	3	T *vs* C 1.28 (0.63-2.59)*	0.5	TT *vs* CC 1.53 (0.63-3.69)*	0.34	(TT+TC) *vs* CC 1.20 (0.64-2.27)*	0.57	1.33 (0.87-2.04)†	0.12
rs763780	4	C *vs* T 1.60 (0.98-2.61)*	0.06	CC *vs* TT 1.42 (1.06-1.91)†	0.02	(CC+CT) *vs* TT 1.26 (1.09-1.46)†	0.002	1.24 (0.90-1.72)†	0.91

*Estimates for random-effects model; †Estimates for fixed-effects model; #p value of Z-test for pooled odds ratio; §Added 0.01 to zero frequency of cell; ORG, generalized odds ratio; ‡p value for Q-test to estimate heterogeneity between studies, p< 0.05 indicates significant heterogeneity

Table 4. Association between the 'A' Allele Carriers of IL-17A SNP rs2275913 and Clinicopathological Features of Gastric Cancer

	Study included	(AA+AG) *vs* GG OR (95% CI)	*p* value#
Location	3		
Cardia		1.01 (0.79-1.28)†	0.95
Noncardia		1.37 (1.16-1.62)*	0.0003
Lauren type	2		
Intestinal		1.20 (0.98-1.45)*	0.07
Diffuse		1.02 (0.73-1.42)*	0.92
Helicobacter pylori infection	3		
H. pylori+		1.61 (1.28-2.02)†	0.0001
H.pylori-		1.08 (0.81-1.43)†	0.59
Differentiation	2		
Poor		1.35 (1.06-1.73)*	0.02
Moderate		1.04 (0.78-1.38)*	0.81
Well		2.42 (1.18-4.97)*	0.02
TNM stage	2		
I-II		1.66 (1.17-2.34)*	0.004
III-IV		0.80 (0.34-1.89)†	0.61

*Estimates for random-effects model; †Estimates for fixed-effects model; #*p* value of Z-test for pooled odds ratio

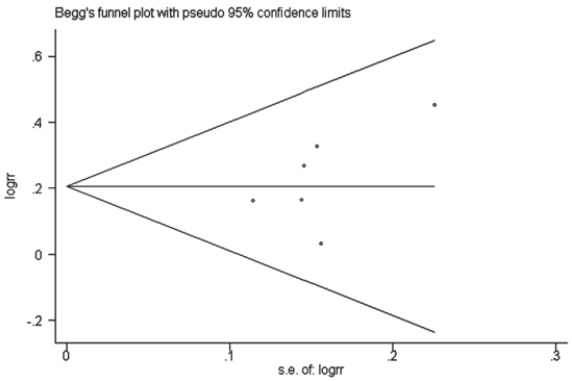

Figure 3. Begg's Funnel Plot of Publication Bias for the Association of the 'A' allele Carriers of IL-17A SNP rs2275913 with the Risk of Gastric Cancer. Each point represents a separate study for the indicated association

any study one by one did not alter the statistical results. Begg's funnel plot and Egger's test were performed to assess publication bias of the literature. The shape of the funnel plot was symmetrical and the statistical results did not show any publication bias (Begg's test: p=0.188, Egger's test: p= 0.263 for rs2275913, Figure 3).

Discussion

Previous reports indicated that chronic inflammation may be involved in the development of gastric cancer, because IL-17A and IL-17F are expressed by Th17 cells which mediate chronic inflammation and various diseases (Park et al., 2005; Hu et al., 2013; Lee et al., 2013). Thus far, some studies have investigated the association between IL-17A and IL-17F polymorphisms and susceptibility to gastric cancer, but the results were unclear. Therefore, we conducted a comprehensive meta-analysis of large samples to provide insight into the relationship between the IL-17A and IL-17F gene SNPs and gastric cancer and obtain a more reliable conclusion.

The findings from the current meta-analysis suggested that the IL-17A gene polymorphism is associated with gastric cancer risk. Importantly, the rs2275913 polymorphism is located in the 5' region near the IL-17A gene which may regulate the gene transcription. Hence, the functions of this SNP need to be further investigated in future studies.

In interpreting the results of the meta-analysis, some limitations should be addressed. First, although we observed a significant association between the IL-17A polymorphism and the risk of gastric cancer in multiple genetic models and stratified analysis by clinicopathological features, the small number of references included in our study precluded a thorough evaluation of publication bias. Second, environmental factors are also important in the development of tumor disease. The potential interactions between genetic and environmental factors may also modify the development of gastric cancer. Failing to consider these factors may influence the effects of IL-17 polymorphisms on the susceptibility to gastric cancer. Third, different genotyping methods may also have a possible effect on the frequency of the allele. Fourth, we have only reviewed published studies, but some unpublished studies, especially negative results, may affect the final conclusion. Fifth, the current data were only from Asian populations. Moreover, the 'A' allele frequency of the rs2275913 polymorphism in Chinese gastric cancer patients is 48.8% and 28.8% in Wu's study and Zhang's study, respectively, suggesting that the diversity in geography, environment, and demography might also cause genetic heterogeneity in different populations even within a country (Pan et al., 2013).

Taken together, despite some limitations, the present meta-analysis provided a more precise estimation of the relationship between the IL-17 gene SNPs and gastric cancer risk compared with the individual studies, which also supports the previous findings (Dai et al., 2014). Furthermore, other possible causative variants in the IL-17 gene should be explored.

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