**Name of Journal:** *World Journal of Gastrointestinal Oncology*

**Manuscript NO:** 51222

**Manuscript Type:** ORIGINAL ARTICLE

***Case Control Study***

**Association between *interleukin-21* gene rs907715 polymorphism and gastric precancerous lesions in a Chinese population**

Wang XQ *et al.* Gene polymorphism with gastric precancerous lesions

Xiao-Qin Wang, Yang Li, Paul D Terry, Wen-Jing Kou, Yue Zhang, Zhao-Zhao Hui, Xiao-Han Ren, Ming-Xu Wang

**Xiao-Qin Wang, Yang Li, Wen-Jing Kou, Yue Zhang, Xiao-Han Ren, Ming-Xu Wang,** Department of Public Health, Xi’an Jiaotong University Health Science Center, Xi’an 710061, Shaanxi Province, China

**Paul D Terry,** Graduate School of Medicine, University of Tennessee Medical Center, Knoxville, TN 37920, United States

**Zhao-Zhao Hui,** The Nethersole School of Nursing, The Chinese University of Hong Kong, Hong Kong, China

**Author contributions:** Wang XQ wrote the manuscript; Wang XQ and Li Y analyzed the data; Li Y, Kou WJ, Zhang Y, Hui ZZ, and Ren XH performed the majority of the experiments; Wang XQ and Wang MX designed the research; Wang MX and Terry PD revised the manuscript.

**Supported by** the Fundamental Research Funds for the Central Universities, No. zdyf2017007; Project of Shaanxi Health and Culture Research Center, No. JKWH2019-Z02.

**Corresponding author: Mingxu Wang, PhD, Professor,** Department of Public Health, Xi’an Jiaotong University Health Science Center, 76 Yanta West Road, Xi’an 710061, Shaanxi Province, China. wangmx601@mail.xjtu.edu.cn

**Received:** September 6, 2019

**Revised:** November 18, 2019

**Accepted:** January 6, 2020

**Published online:**

**Abstract**

BACKGROUND

The single nucleotide polymorphisms of interleukin-21(*IL-21*) gene were confirmed to be related to various diseases, but no studies have examined the possible role of *IL-21* single nucleotide polymorphisms (SNPs) (rs907715, rs2221903, and rs12508721) in gastric precancerous lesions.

AIM

To explore the associations between SNPs of *IL-21* gene (rs907715, rs2221903, and rs12508721) and gastric precancerous lesions in a Chinese population.

METHODS

Three SNPs of *IL-21* were genotyped using polymerase chain reaction–ligase detection reaction in 588 cases and 290 healthy controls from May 2013 to December 2016 in northwestern China. Gastric precancerous lesions were confirmed by endoscopic examination and categorized as non-atrophic gastritis, atrophic gastritis, and intestinal metaplasia. Descriptive statistic and logistic regression were used for data analyses.

RESULTS

*IL-21* rs907715 genotype CC and C frequencies were higher in in patients with gastric precancerous lesions than in the controls (OR = 1.59, 95%CI: 1.06-2.38, *P* = 0.013; OR = 1.28, 95%CI: 1.01-2.22, *P* = 0.044, respectively) after adjusting for confounding factors. For SNP rs907715 in intestinal metaplasia patients, significant differences between cases and controls were observed in the frequencies of genotype CC and C (OR = 1.92, 95%CI: 1.24-2.98, *P* = 0.004; OR = 1.53, 95%CI: 1.04-2.24, *P* = 0.028, respectively); for non-atrophic gastritis and atrophic gastritis patients, the CC and C genotypes showed no significant association with risk in all models. No association between either rs2221903 or rs12508721 and gastric precancerous lesions was found in the present study. In the haplotype analysis, the TC haplotype (rs907715 and rs12508721) and TT haplotype (rs2221903 and rs907715) were more frequent in the case group than control group (*P* < 0.05).

CONCLUSION

Our findings indicate that SNP rs907715 of *IL-21* gene is associated with gastric precancerous lesions. The TC haplotype (rs907715 and rs12508721) and TT haplotype (rs2221903 and rs907715) increased the risk of gastric precancerous lesions. If confirmed, these findings will shed light on the etiology of precancerous lesions.

**Key words:** *Interleukin-21* gene; Single nucleotide polymorphisms; rs907715; Gastric precancerous lesions; Intestinal metaplasia

Wang XQ, Li Y, Terry PD, Kou WJ, Zhang Y, Hui ZZ, Ren XH, Wang MX. Association between *Interleukin-21* gene rs907715 polymorphism and gastric precancerous lesions in a Chinese population. *World J Gastrointest Oncol* 2020; In press

**Core tip:** This study investigated the associations between single nucleotide polymorphisms of interleukin-21(*IL-21*) gene (rs907715, rs2221903 and rs12508721) and gastric precancerous lesions in a Chinese population. The results showed an association between *IL-21* rs907715 polymorphism and gastric precancerous lesions. *IL-21* rs907715 genotype CC and C frequencies were higher in patients with gastric precancerous lesions than in the controls. Single nucleotide polymorphism rs907715 increased in CC and C genotypes were associated with intestinal metaplasia patients when examined separately. These findings may help clarify the etiology of gastric cancer.

**INTRODUCTION**

Gastric cancer (GC) is the fifth most common malignancy worldwide, and ranks second in incidence and mortality among all malignancies in China[1,2]. GC is considered to be a multistep progression from non-atrophic gastritis (NAG), atrophic gastritis (AG), intestinal metaplasia (IM), dysplasia, to gastric adenocarcinoma[3]. The worldwide prevalences of AG and IM were 33% and 25% respectively[4]. As the specific recognizable stages of the precancerous cascade[5], gastric precancerous lesions can increase the risk of GC[6,7], so clarifying the etiology of gastric precancerous lesions is of great significance in preventing the development of GC[8]. Multiple factors contribute to the occurrence and development of gastric precancerous lesions, including environmental factors, such as *Helicobacter pylori* infection[9,10], high salt intake[11,12], alcohol consumption[12], and smoking status[13]. Some studies have explored genetic risk factors for precancerous lesions such as *interleukin (IL)-1*, *IL-8*, *IL-10*, and *IL-22*[14-18], but less attention has been given to *IL-21*.

*IL-21* is an immune modulatory cytokine produced mainly by activated CD4+T cells and natural killer (NK) cells, and has multiple effects on innate and adaptive immune responses[19]. The activity of *IL-21* is mediated via binding to a compound receptor consisting of *IL-21R* and γ chain[20,21], and the biological functions of *IL-21* include promoting T-cell proliferation, stimulating B-cell differentiation, and enhancing NK-cell activation[22,23]. *IL-21* plays important roles in inflammatory, antiviral, and antitumor responses[24]. Single nucleotide polymorphisms (SNPs) of the *IL-21* gene can change the expression level of mRNA, resulting in a change in protein expression or autoantibody production[25]. SNPs of *IL-21* have been associated with various diseases of the immune system including systemic lupus erythematosus[26], Graves’ disease[27], rheumatoid arthritis[28], and hepatitis B virus (HBV) infection[29]. Several SNPs of *IL-21* (rs907715, rs2221903 and rs12508721) have also been associated with the susceptibility to cancer[30-33]. For example, SNPs rs907715 and rs2221903 reduce the susceptibility to non-small cell lung cancer[30], and SNP rs12508721 is related to thyroid cancer[31], breast cancer[32] and HBV-related hepatocellular carcinoma[33]. Previous studies have found that *IL-21* may be associated with the risk of gastric precancerous lesions[34,35]. However, no studies have examined the possible role of *IL-21* SNPs (rs907715, rs2221903 and rs12508721) in gastric precancerous lesions. Therefore, the present study explored associations between SNPs of *IL-21* (rs907715, rs2221903 and rs12508721) and risk of gastric precancerous lesions in a northwestern Chinese population.

**MATERIALS AND METHODS**

***Subjects***

This study was conducted from May 2014 to December 2016 in hospitals from three cities (Yulin, North; Xi'an, Middle; Hanzhong, South) in Shaanxi Province, China (Figure 1). Men and women with gastrointestinal symptoms requiring upper endoscopy examination were screened for study eligibility. Individuals diagnosed with GC were excluded, while a total of 1674 subjects who had undergone upper gastrointestinal endoscopy, completed pathological and 24-hour urine testing were included. The medical records of all subjects were reviewed retrospectively.

Of the eligible and willing subjects, 588 with NAG, AG, or IM (cases) and 290 without any diagnosis of gastric diseases or *H. pylori* infection (controls) were enrolled. This study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the Institutional Review Board of Xi’an Jiaotong University Health Science Center. Informed consent was obtained from all subjects.

***Data measurements and collection***

Demographic information was obtained from subjects’ medical records including age, gender, smoking status, drinking status, height, and weight. For smoking status and drinking status, subjects were dichotomized as “yes” or “no”. Body mass index was calculated as weight in kilograms divided by height in meters squared.

Daily salt intake was determined by 24-hour urine sodium excretion and was dichotomized as “high salt” and “non-high salt” according to the median of the controls (representing the general population). Subjects were asked to excrete and discard their first urine at 7 a.m. and to collect all urine over the following 24-hours, including the next day first urine at 7 a.m. Total volumes of the collection were measured. Urinary sodium levels were measured by the ion selective electrode method using by Olympus AU 680 autoanalyser.

In this study, NAG, AG, and IM were diagnosed by endoscopic findings and based on updated Sydney system criteria[36] and Atrophy Club criteria[37]. The serum *H. pylori* IgG antibody test was performed by an enzyme-linked immunosorbent assay on the same day of endoscopy. IgG values ≥ 10 U/mL were considered as “*H. pylori* infection” and < 10 U/mL as negative results[38].

***Genotyping***

Three SNPs (rs907715, rs2221903, and rs12508721) of IL-21 were genotyped in cases and controls. Genomic DNA was extracted from 5 mL peripheral blood samples using the Blood DNA Kit (Tiangen, Beijing, China), and stored at -80°C until subsequent assay. SNPs were genotyped using the polymerase chain reaction (PCR)–ligase detection reaction method using assay-on-demand probes and primers: C\_8949748\_10 for rs907715, C\_16167441\_10 for rs2221903, C\_1597500\_10 for rs12508721. The forward and reverse primers are shown in Table 1. All primers were designed using the Primer3 program (http://frodo.wi.mit. edu/cgi-bin/primer3/primer3\_www.cgi). The reaction was performed in a total volume of 20 µL, containing genomic DNA (1 µL), buffer (2 µL), MgCl2 (0.6 µL), dNTPs (2 µL), *Taq* polymerase (0.2 µL), 2 µL of each primer, and 12.2 µL ddH2O. PCR conditions were as follows: denaturation at 95 °C for 2 min, 94 °C for 30 s; annealing at 56 °C for 90 s; extension at 40 cycles of 65 °C for 30 s, and a final extension at 65 °C for 10 min. Following amplification, PCR products were submitted for DNA sequencing.

Trizol was used for extraction of mRNA from six intestinal epithelium tissues according to the manufacturer’s instructions. Quantification of mRNA of rs907715 was performed using BioEasy SYBR Green Real Time PCR Kit in a 20 μL reaction volume, containing SYBR Green Master Mix (10 μL), PCR Forward Primer (0.8 μL), cDNA (2 μL), ROX (0.4 μL) and nuclease-free water (6 μL). Extension was performed under the following conditions: initial denaturation at 95 °C for 5 min, followed by 40 cycles at 95 °C for 5 s and 60 °C for 34 s. All reactions were performed in duplicate. Using the 2−ΔΔCt method[39] to calculate the relative mRNA expression levels.

***Statistical analysis***

Descriptive statistics were used to describe demographic characteristics of all subjects in our study. Genotype frequencies of three SNPs (rs907715, rs2221903, and rs12508721) were obtained by statistical description, and Hardy-Weinberg equilibrium was analyzed using the chi-squared goodness of fit test. Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) of gastric precancerous lesions for genotype, controlling for demographic and lifestyle factors (age, gender, body mass index, drinking status, smoking status, daily salt intake and region). Distribution normality of *IL-21* mRNA expression was assessed using the Kolmogorov–Smirnov test, differences among three genotypes were measured using an independent sample Student’s *t*-test. All analyses were performed with SPSS 22.0 software (IBM, Chicago, IL, United States). A two-tailed *P* < 0.05 was considered statistically significant.

**RESULTS**

***Demographic and lifestyle associations***

A total of 1674 subjects were included in the study, aged 26 to 88 with a mean age of 49.8 (SD = 11.4). The incidences of NAG, AG, and IM were 6.9%, 6.3%. and 21.9%, respectively (Table 2). The NAG incidence in the south region was higher than that in other two regions, while AG and IM incidences in middle region were higher than those in other two regions.

High salt intake was associated with an increased risk of NAG (OR = 2.58, 95%CI: 1.21-4.88, *P* = 0.011) (Table 3); *H. pylori* infection was correlated with decreased risk of AG (OR = 0.39, 95% 95%CI: 0.65-0.99, *P* = 0.041); and smoking was related to increased risk of NAG (OR = 2.15, 95%CI: 1.19-4.44, *P* = 0.015) and IM (OR = 1.97, 95%CI: 1.40-2.58, *P* = 0.005). Compared with the south region, subjects in the middle region had a lower risk of NAG (OR = 0.33, 95%CI: 0.28-0.51, *P* = 0.009) and a higher risk of IM (OR = 2.95, 95%CI: 1.45-4.33, *P* = 0.007); subjects in the north region had a lower risk of AG (OR = 0.33, 95%CI: 0.21-0.83, *P* = 0.010).

***Association of IL-21 gene polymorphisms and gastric precancerous lesions***

The genotype distributions of each group were consistent with Hardy-Weinberg equilibrium (*P* > 0.05) (Table 4). In univariate analyses, differences in the distribution frequency of rs907715 genotypes CC and C between cases and controls were statistically significant (OR = 1.77, 95%CI: 1.19-2.63, *P* = 0.005; OR = 1.43, 95%CI: 1.02-2.01, *P =* 0.039, respectively). Results were similar after adjusting for confounding factors (OR = 1.59, 95%CI: 1.06-2.38, *P* = 0.013; OR = 1.28, 95%CI: 1.01-2.22, *P =* 0.044, respectively). Results were also similar for IM when examined separately (OR = 1.92, 95%CI: 1.24-2.98, *P =* 0.004; OR = 1.53, 95%CI: 1.04-2.24, *P =* 0.028, respectively). The distribution frequencies of genotype CC and C were not statistically different between cases and controls in all models.

Analyses of rs907715 mRNA expression in intestinal epithelium tissue from six subjects with IM showed significant differences between CC genotype and TT genotype (*P* < 0.001), CC genotype and CT genotype (*P* < 0.01) (Figure 2). Similarly, rs907715 mRNA expression levels in six NAG tissues and six atrophic gastritis tissues were conducted. For NAG tissues, the expression level of rs907715 CC genotype was significantly different from that among the rs907715 CT genotype (*P* < 0.05) and TT genotype (*P* < 0.01) (Figure 3); for atrophic gastritis tissues, the expression level showed significant difference between rs907715 CC genotype and TT genotype (*P* < 0.05) (Figure 4).

***Haplotype Analyses***

The results showed that the TC haplotype (rs907715 and rs12508721) was significantly associated with AG and IM (OR = 3.91, *P =* 0.003; OR = 2.02, *P =* 0.004, respectively), and it appeared to be a risk haplotype; the TT haplotype (rs2221903 and rs907715) was significantly associated with IM (OR = 1.44, *P =* 0.023) and it appeared to be a risk haplotype (Table 5).

**DISCUSSION**

Our results suggested that rs907715 genotypes CC and C confer increased susceptibility to IM and total gastric precancerous lesions, whereas no association was found for rs2221903 or rs12508721. Because we are not aware of any previous study that directly addressed these associations, our findings should be interpreted cautiously.

Regarding rs907715, Liu *et al*[30], for example, reported that genotype AA and A allele of rs907715 were associated with the decreased susceptibility to non-small cell lung cancer. Xiao *et al*[31] revealed that the G allele of rs907715 increased the susceptibility to Graves’ disease. Moreover, a case-control study found that serum *IL-21* levels in HBV patients with rs907715 genotype AA were lower than those in patients with genotype AG/GG; this genotype was independently related to sustained virological response[40]. A meta-analysis showed that the genotype distribution of *IL-21* rs907715 was significantly different between systemic lupus erythematosus patients and healthy controls in all genetic models[26]. All of these findings suggest that rs907715 of *IL-21* may to some extent exert effects on antitumor, antiviral and/or inflammatory processes. However, other studies have shown no association between rs907715 and thyroid cancer[31] and breast cancer[32]. Hence, the associations we observed in our study should be addressed in future studies.

The *IL-21* gene is located on human chromosome 4q26-27 and plays an important role in anti-tumor immunopathology[41]. Previous studies have found that *IL-21* is overexpressed in *H. pylori*-infected gastric mucosa[42], and is correlated with the occurrence and development of gastritis[34,35]. Moreover, studies have found that the concentration of *IL-21* is increased in both tissue and serum of GC patients[43,44]. Thus, IL-21 may play a role in the development and progression of GC and gastritis-related diseases. Previous evidence has shown that SNP rs907715 is associated with increased *IL-21* transcription and expression[40,45]. The rs907715, locating in the third intron of *IL-21* gene, may be a surrogate marker for mutations with functional consequences[25]. SNPs including rs907715 may be in linkage disequilibrium with a variant correlated with mRNA translation, thereafter may lead to the change of protein expression[27]. Therefore, SNP rs907715 of *IL-21* gene may alter the mRNA expression levels and regulate the function of *IL-21*. This suggested that rs907715 may be related to the risk of gastric precancerous lesions by influencing the activities of *IL-21*.

The data regarding TC haplotype frequency (rs907715 and rs12508721) in AG and IM patients compared to controls showed that this haplotype may be a risk for gastric precancerous lesions. Similarly, the TT haplotype frequency (rs2221903 and rs907715) in IM patient compared to controls showed that this haplotype may be a risk for IM. This result suggested that the two haplotypes, according to the *IL-21* polymorphisms, might be the important genetic factors for susceptibility to gastric precancerous lesions.

The present study selected subjects in three cities from north to south in Shaanxi province, which enhanced the power of population representation and made our results more credible. However, this study also has several limitations. First, all cases and controls were selected from participants experiencing upper gastrointestinal symptoms, which may cause a potential selection bias and increase the positive results of the study. Second, controls were screened from subjects with non-*H. pylori* infection and non-precancerous lesions to represent the general population, this led to a mismatched number of cases and controls, which may weaken the testing effectiveness. Third, this study was a case-control study and unable to draw a causal relationship.

In conclusion, our study found that SNP rs907715 was associated with gastric precancerous lesions, and the TC haplotype (rs907715 and rs12508721) and TT haplotype (rs2221903 and rs907715) increased the risk of gastric precancerous lesions, which may help clarify the etiology of GC. Further studies are required to elucidate the roles of rs907715 in development of gastric precancerous lesions at a molecular level, which may provide new targets for therapeutic interventions.

**ARTICLE HIGHLIGHTS**

***Research background***

Previous studies have found that interleukin-21(*IL-21*) may be associated with the risk of gastric precancerous lesions, and single nucleotide (SNPs) of the *IL-21* gene are associated with various diseases or cancer. Clarifying the possible role of *IL-21* SNPs (rs907715, rs2221903 and rs12508721) in gastric precancerous lesions is of great significance in preventing the development of gastric cancer.

***Research motivation***

However, no studies have examined the possible role of *IL-21* SNPs (rs907715, rs2221903 and rs12508721) in gastric precancerous lesions.

***Research objectives***

Therefore, the present study explored the associations between SNPs of *IL-21* (rs907715, rs2221903 and rs12508721) and risk of gastric precancerous lesions in a north western Chinese population, which may help clarify the etiology of gastric cancer and provide new targets for therapeutic interventions.

***Research methods***

Gastric precancerous lesions were confirmed by endoscopic examination and categorized as non-atrophic gastritis, atrophic gastritis, and intestinal metaplasia. Three SNPs of *IL-21* (rs907715, rs2221903 and rs12508721) were genotyped using polymerase chain reaction–ligase detection reaction in 588 cases and 290 healthy controls. Descriptive statistic and logistic regression were used for data analyses.

***Research results***

We found an association between *IL-21* rs907715 polymorphism and gastric precancerous lesions. *IL-21* rs907715 genotype CC and C frequencies in patients with gastric precancerous lesions were higher than in controls. SNP rs907715 increased in CC and C genotypes were associated with intestinal metaplasia patients when examined separately. However, the exact role of rs907715 in development of gastric precancerous lesions at a molecular level remains to be studied.

***Research conclusions***

In conclusion, our findings indicate that SNP rs907715 of *IL-21* gene is associated with gastric precancerous lesions.

***Research perspectives***

If confirmed by other studies, the results of our study suggest that *IL-21* rs907715 polymorphisms may shed light on the etiology of precancerous lesions.

**REFERENCES**

1 **American Cancer Society.** Global Cancer Facts & Figures 4th Edition. Atlanta: American Cancer Society 2018; 33-34. Available from: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/global-cancer-facts-and-figures/global-cancer-facts-and-figures-4th-edition.pdf.

2 **Chen W**, Zheng R, Zhang S, Zeng H, Xia C, Zuo T, Yang Z, Zou X, He J. Cancer incidence and mortality in China, 2013. *Cancer Lett* 2017; **401**: 63-71 [PMID: 28476483 DOI: 10.1016/j.canlet.2017.04.024]

3 **Correa P**. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992; **52**: 6735-6740 [PMID: 1458460]

4 **Marques-Silva L**, Areia M, Elvas L, Dinis-Ribeiro M. Prevalence of gastric precancerous conditions: a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2014; **26**: 378-387 [PMID: 24569821 DOI: 10.1097/MEG.0000000000000065]

5 **Correa P**, Piazuelo MB. The gastric precancerous cascade. *J Dig Dis* 2012; **13**: 2-9 [PMID: 22188910 DOI: 10.1111/j.1751-2980.2011.00550.x]

6 **Lage J**, Uedo N, Dinis-Ribeiro M, Yao K. Surveillance of patients with gastric precancerous conditions. *Best Pract Res Clin Gastroenterol* 2016; **30**: 913-922 [PMID: 27938786 DOI: 10.1016/j.bpg.2016.09.004]

7 **Zhang L**, Liu Y, You P, Feng G. Occurrence of gastric cancer in patients with atrophic gastritis during long-term follow-up. *Scand J Gastroenterol* 2018; **53**: 843-848 [PMID: 29911441 DOI: 10.1080/00365521.2018.1477987]

8 **Dinis-Ribeiro M**, Areia M, de Vries AC, Marcos-Pinto R, Monteiro-Soares M, O'Connor A, Pereira C, Pimentel-Nunes P, Correia R, Ensari A, Dumonceau JM, Machado JC, Macedo G, Malfertheiner P, Matysiak-Budnik T, Megraud F, Miki K, O'Morain C, Peek RM, Ponchon T, Ristimaki A, Rembacken B, Carneiro F, Kuipers EJ; European Society of Gastrointestinal Endoscopy; European Helicobacter Study Group; European Society of Pathology; Sociedade Portuguesa de Endoscopia Digestiva. Management of precancerous conditions and lesions in the stomach (MAPS): guideline from the European Society of Gastrointestinal Endoscopy (ESGE), European Helicobacter Study Group (EHSG), European Society of Pathology (ESP), and the Sociedade Portuguesa de Endoscopia Digestiva (SPED). *Endoscopy* 2012; **44**: 74-94 [PMID: 22198778 DOI: 10.1055/s-0031-1291491]

9 **Watari J**, Chen N, Amenta PS, Fukui H, Oshima T, Tomita T, Miwa H, Lim KJ, Das KM. Helicobacter pylori associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. *World J Gastroenterol* 2014; **20**: 5461-5473 [PMID: 24833876 DOI: 10.3748/wjg.v20.i18.5461]

10 **Shimizu T**, Chiba T, Marusawa H. Helicobacter pylori-Mediated Genetic Instability and Gastric Carcinogenesis. *Curr Top Microbiol Immunol* 2017; **400**: 305-323 [PMID: 28124159 DOI: 10.1007/978-3-319-50520-6\_13]

11 **Song JH**, Kim YS, Heo NJ, Lim JH, Yang SY, Chung GE, Kim JS. High Salt Intake Is Associated with Atrophic Gastritis with Intestinal Metaplasia. *Cancer Epidemiol Biomarkers Prev* 2017; **26**: 1133-1138 [PMID: 28341758 DOI: 10.1158/1055-9965.EPI-16-1024]

12 **Thapa S**, Fischbach LA, Delongchamp R, Faramawi MF, Orloff M. Association between Dietary Salt Intake and Progression in the Gastric Precancerous Process. *Cancers (Basel)* 2019; **11** [PMID: 30987215 DOI: 10.3390/cancers11040467]

13 **Ferro A**, Morais S, Rota M, Pelucchi C, Bertuccio P, Bonzi R, Galeone C, Zhang ZF, Matsuo K, Ito H, Hu J, Johnson KC, Yu GP, Palli D, Ferraroni M, Muscat J, Malekzadeh R, Ye W, Song H, Zaridze D, Maximovitch D, Aragonés N, Castaño-Vinyals G, Vioque J, Navarrete-Muñoz EM, Pakseresht M, Pourfarzi F, Wolk A, Orsini N, Bellavia A, Håkansson N, Mu L, Pastorino R, Kurtz RC, Derakhshan MH, Lagiou A, Lagiou P, Boffetta P, Boccia S, Negri E, La Vecchia C, Peleteiro B, Lunet N. Tobacco smoking and gastric cancer: meta-analyses of published data versus pooled analyses of individual participant data (StoP Project). *Eur J Cancer Prev* 2018; **27**: 197-204 [PMID: 29595756 DOI: 10.1097/CEJ.0000000000000401]

14 **Chiurillo MA**. Role of gene polymorphisms in gastric cancer and its precursor lesions: current knowledge and perspectives in Latin American countries. *World J Gastroenterol* 2014; **20**: 4503-4515 [PMID: 24782603 DOI: 10.3748/wjg.v20.i16.4503]

15 **Cai M**, Dai S, Chen W, Xia C, Lu L, Dai S, Qi J, Wang M, Wang M, Zhou L, Lei F, Zuo T, Zeng H, Zhao X. Environmental factors, seven GWAS-identified susceptibility loci, and risk of gastric cancer and its precursors in a Chinese population. *Cancer Med* 2017; **6**: 708-720 [PMID: 28220687 DOI: 10.1002/cam4.1038]

16 **Liu S**, Liu JW, Sun LP, Gong YH, Xu Q, Jing JJ, Yuan Y. Association of IL10 gene promoter polymorphisms with risks of gastric cancer and atrophic gastritis. *J Int Med Res* 2018; **46**: 5155-5166 [PMID: 30205739 DOI: 10.1177/0300060518792785]

17 **Negovan A**, Iancu M, Fülöp E, Bănescu C. *Helicobacter pylori* and cytokine gene variants as predictors of premalignant gastric lesions. *World J Gastroenterol* 2019; **25**: 4105-4124 [PMID: 31435167 DOI: 10.3748/wjg.v25.i30.4105]

18 **Hnatyszyn A**, Wielgus K, Kaczmarek-Rys M, Skrzypczak-Zielinska M, Szalata M, Mikolajczyk-Stecyna J, Stanczyk J, Dziuba I, Mikstacki A, Slomski R. Interleukin-1 gene polymorphisms in chronic gastritis patients infected with Helicobacter pylori as risk factors of gastric cancer development. *Arch Immunol Ther Exp (Warsz)* 2013; **61**: 503-512 [PMID: 23995914 DOI: 10.1007/s00005-013-0245-y]

19 **Long D**, Chen Y, Wu H, Zhao M, Lu Q. Clinical significance and immunobiology of IL-21 in autoimmunity. *J Autoimmun* 2019; **99**: 1-14 [PMID: 30773373 DOI: 10.1016/j.jaut.2019.01.013]

20 **Habib T**, Senadheera S, Weinberg K, Kaushansky K. The common gamma chain (gamma c) is a required signaling component of the IL-21 receptor and supports IL-21-induced cell proliferation via JAK3. *Biochemistry* 2002; **41**: 8725-8731 [PMID: 12093291 DOI: 10.1021/bi0202023]

21 **Spolski R**, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu Rev Immunol* 2008; **26**: 57-79 [PMID: 17953510 DOI: 10.1146/annurev.immunol.26.021607.090316]

22 **Al-Chami E**, Tormo A, Khodayarian F, Rafei M. Therapeutic utility of the newly discovered properties of interleukin-21. *Cytokine* 2016; **82**: 33-37 [PMID: 26748727 DOI: 10.1016/j.cyto.2015.12.018]

23 **Davis ID**, Skak K, Smyth MJ, Kristjansen PE, Miller DM, Sivakumar PV. Interleukin-21 signaling: functions in cancer and autoimmunity. *Clin Cancer Res* 2007; **13**: 6926-6932 [PMID: 18056166 DOI: 10.1158/1078-0432.CCR-07-1238]

24 **Spolski R**, Leonard WJ. Interleukin-21: a double-edged sword with therapeutic potential. *Nat Rev Drug Discov* 2014; **13**: 379-395 [PMID: 24751819 DOI: 10.1038/nrd4296]

25 **Liu J**, Cen H, Ni J, Zhang M, Li P, Yang XK, Leng RX, Pan HF, Ye DQ. Association of IL-21 polymorphisms (rs907715, rs2221903) with susceptibility to multiple autoimmune diseases: a meta-analysis. *Autoimmunity* 2015; **48**: 108-116 [PMID: 25074442 DOI: 10.3109/08916934.2014.944262]

26 **Qi JH**, Qi J, Xiang LN, Nie G. Association between IL-21 polymorphism and systemic lupus erythematosus: a meta-analysis. *Genet Mol Res* 2015; **14**: 9595-9603 [PMID: 26345892 DOI: 10.4238/2015.August.14.22]

27 **Zeng H**, Yan H, Zhang Z, Fang W, Ding R, Huang L, Chen M, Zhang J. Association between IL-21 gene rs907715 polymorphisms and Graves' disease in a Southern Chinese population. *Exp Ther Med* 2014; **8**: 213-218 [PMID: 24944624 DOI: 10.3892/etm.2014.1707]

28 **Malinowski D**, Paradowska-Gorycka A, Safranow K, Pawlik A. Interleukin-21 gene polymorphism rs2221903 is associated with disease activity in patients with rheumatoid arthritis. *Arch Med Sci* 2017; **13**: 1142-1147 [PMID: 28883856 DOI: 10.5114/aoms.2017.68945]

29 **Feng L**, Li B, Li B. Meta-analysis of correlation between rs907715, rs2221903 and rs12508721 polymorphisms in IL-21 and susceptibility to Hepatitis B. *Minerva Med* 2019 [PMID: 31282133 DOI: 10.23736/S0026-4806.19.06167-6]

30 **Liu L**, Shi F, Li S, Liu X, Wei L, Zhang J, Ju X, Yu J. IL-21 polymorphisms rs907715 and rs2221903 are associated with decreased non-small cell lung cancer susceptibility. *Int J Clin Exp Med* 2015; **8**: 19460-19465 [PMID: 26770592]

31 **Xiao M**, Hu S, Tang J, Zhang L, Jiang H. Interleukin (IL)-21 promoter polymorphism increases the risk of thyroid cancer in Chinese population. *Gene* 2014; **537**: 15-19 [PMID: 24389496 DOI: 10.1016/j.gene.2013.12.050]

32 **You Y**, Deng J, Zheng J, Hu M, Li N, Wu H, Li W, Lu J, Zhou Y. IL-21 gene polymorphism is associated with the prognosis of breast cancer in Chinese populations. *Breast Cancer Res Treat* 2013; **137**: 893-901 [PMID: 23288348 DOI: 10.1007/s10549-012-2401-1]

33 **Zhang QX**, Li SL, Yao YQ, Li TJ. Association between interleukin-21 gene polymorphisms (rs12508721) and HBV-related hepatocellular carcinoma. *Int J Immunogenet* 2016; **43**: 151-158 [PMID: 27122304 DOI: 10.1111/iji.12263]

34 **Bagheri N**, Azadegan-Dehkordi F, Shirzad M, Zamanzad B, Rahimian G, Taghikhani A, Rafieian-Kopaei M, Shirzad H. Mucosal interleukin-21 mRNA expression level is high in patients with Helicobacter pylori and is associated with the severity of gastritis. *Cent Eur J Immunol* 2015; **40**: 61-67 [PMID: 26155185 DOI: 10.5114/ceji.2015.50835]

35 **Nishiura H**, Iwamoto S, Kido M, Aoki N, Maruoka R, Ikeda A, Chiba T, Watanabe N. Interleukin-21 and tumor necrosis factor-α are critical for the development of autoimmune gastritis in mice. *J Gastroenterol Hepatol* 2013; **28**: 982-991 [PMID: 23425147 DOI: 10.1111/jgh.12144]

36 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181 [PMID: 8827022 DOI: 10.1097/00000478-199610000-00001]

37 **Rugge M**, Correa P, Dixon MF, Fiocca R, Hattori T, Lechago J, Leandro G, Price AB, Sipponen P, Solcia E, Watanabe H, Genta RM. Gastric mucosal atrophy: interobserver consistency using new criteria for classification and grading. *Aliment Pharmacol Ther* 2002; **16**: 1249-1259 [PMID: 12144574 DOI: 10.1046/j.1365-2036.2002.01301.x]

38 **Wang XQ**, Yan H, Terry PD, Wang JS, Cheng L, Wu WA, Hu SK. Interaction between dietary factors and Helicobacter pylori infection in noncardia gastric cancer: a population-based case-control study in China. *J Am Coll Nutr* 2012; **31**: 375-384 [PMID: 23529995 DOI: 10.1080/07315724.2012.10720447]

39 **Livak KJ**, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; **25**: 402-408 [PMID: 11846609 DOI: 10.1006/meth.2001.1262]

40 **Wang X**, Xu ZQ, Fu JJ, Cheng LW, Li Y, Li L, Pan XC. Role of interleukin-21 and interleukin-21 receptor polymorphisms in the treatment of HBeAg-positive chronic hepatitis B patients with peginterferon. *Medicine (Baltimore)* 2018; **97**: e10891 [PMID: 29879024 DOI: 10.1097/MD.0000000000010891]

41 **Yao JY**, Chao K, Li MR, Wu YQ, Zhong BH. Interleukin-21 gene polymorphisms and chronic hepatitis B infection in a Chinese population. *World J Gastroenterol* 2015; **21**: 4232-4239 [PMID: 25892873 DOI: 10.3748/wjg.v21.i14.4232]

42 **Caruso R**, Fina D, Peluso I, Fantini MC, Tosti C, Del Vecchio Blanco G, Paoluzi OA, Caprioli F, Andrei F, Stolfi C, Romano M, Ricci V, MacDonald TT, Pallone F, Monteleone G. IL-21 is highly produced in Helicobacter pylori-infected gastric mucosa and promotes gelatinases synthesis. *J Immunol* 2007; **178**: 5957-5965 [PMID: 17442980 DOI: 10.4049/jimmunol.178.9.5957]

43 **Su Z**, Sun Y, Zhu H, Liu Y, Lin X, Shen H, Chen J, Xu W, Xu H. Th17 cell expansion in gastric cancer may contribute to cancer development and metastasis. *Immunol Res* 2014; **58**: 118-124 [PMID: 24402773 DOI: 10.1007/s12026-013-8483-y]

44 **Meng X**, Yu X, Dong Q, Xu X, Li J, Xu Q, Ma J, Zhou C. Distribution of circulating follicular helper T cells and expression of interleukin-21 and chemokine C-X-C ligand 13 in gastric cancer. *Oncol Lett* 2018; **16**: 3917-3922 [PMID: 30128008 DOI: 10.3892/ol.2018.9112]

45 **Wang H**, Wang M, Feng Z, Chen L, Gao L, Li Q, Zhang L, Ma J. Functional interleukin-21 polymorphism is a protective factor of diffuse large B-cell lymphoma. *DNA Cell Biol* 2014; **33**: 775-780 [PMID: 25126827 DOI: 10.1089/dna.2014.2559]

**Footnotes**

**Institutional review board statement:** This study was approved by the Institutional Review Board of Health Science Center of Xi’an Jiaotong University.

**Informed consent statement:** Informed consent was obtained from all study participants.

**Conflict-of-interest statement:** The authors declare no conflicts of interest.

**Data sharing statement:** No additional data are available.

**STROBE Statement:** The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited Manuscript

**Peer-review started:** September 6, 2019

**First decision:** October 14, 2019

**Article in press:**

**Specialty type:** Oncology

**Country of origin:** China

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): 0

Grade C (Good): C, C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Kaabachi W, Raffaniello R **S-Editor:** Wang YQ **L-Editor:** Filipodia **E-Editor:**

**Figure Legends**



**Figure 1** **Location map of three selected cities in Shaanxi province, China.**



**Figure 2** **Interleukin-21 mRNA expression level in six intestinal epithelium tissues among three rs907715 genotypes.**



**Figure 3** **Interleukin-21 mRNA expression level in six non-atrophic gastritis tissues among three rs907715 genotypes.**



**Figure 4** **Interleukin-21 mRNA expression level in six atrophic gastritis tissues among three rs907715 genotypes.**

**Table 1 Probe primary information for genotyping *interleukin-21* gene polymorphisms**

|  |  |  |
| --- | --- | --- |
| **Primer** | **Type** | **Primer sequences, 5**′**→3**′ |
| rs907715 | F | 5′-ATAGATGAGGAAAGTGAGATC-3′ |
| R | 5′- CTTTGCTTATTTGATATATTCC-3′ |
| rs2221903 | F | 5′-GGACCACATATTGCCAG ACAC-3′ |
| R | 5′-GACACTGACGCCCATATTGAT-3′ |
| rs12508721 | F | 5′-ATGGGACTAAAGT CAAGGTG-3′ |
| R | 5′-AGATGGCTTCTAGAGTCTGG-3′ |

**Table** **2** **Demographic and lifestyle characteristics of participants in different regions**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Characteristic** | **North** | **Middle** | **South** | **Total** |
| ***n =* 742** | **44.3 (%)** | ***n =* 488** | **29.2 (%)** | ***n =* 444** | **26.5 (%)** | ***n =* 1674** | **100 (%)** |
| Gender |  |  |  |  |  |  |  |  |
| Female | 464 | 62.5 | 128 | 26.2 | 136 | 30.6 | 728 | 43.5 |
| Male | 278 | 37.5 | 360 | 73.8 | 308 | 69.4 | 946 | 56.5 |
| BMI | 742 | 20.0 ± 3.2 | 488 | 20.6 ± 2.8 | 444 | 20.3 ± 2.2 | 1674 | 20.6 ± 2.0 |
| Age in yr | 742 | 52.9 ± 15.1 | 488 | 46.3 ± 12.8 | 444 | 47.5 ± 15.0 | 1674 | 49.8 ± 11.4 |
| < 40 | 110 | 36.2 ± 5.4 | 174 | 36.9 ± 5.2 | 76 | 36.7 ± 3.9 | 360 | 35.8 ± 2.9 |
| 40-49 | 220 | 45.1 ± 4.2 | 202 | 44.7 ± 4.5 | 288 | 44.8 ± 2.1 | 710 | 45.5 ± 2.0 |
| 50-59 | 150 | 54.6 ± 6.1 | 28 | 51.8 ± 1.9 | 8 | 54.4 ± 2.5 | 186 | 54.2 ± 2.0 |
| ≥ 60 | 262 | 66.1 ± 5.0 | 84 | 68.3 ± 11.1 | 72 | 77.0 ± 10.4 | 418 | 68.0 ± 8.4 |
| Lifestyle |  |  |  |  |  |  |  |  |
| High salt | 230 | 31.0 | 112 | 23.0 | 56 | 12.6 | 398 | 23.8 |
| Smoking | 314 | 42.3 | 142 | 29.1 | 318 | 71.6 | 774 | 46.2 |
| Drinking | 286 | 38.5 | 116 | 23.8 | 184 | 41.4 | 586 | 35.0 |
| Clinical diagnosis |  |  |  |  |  |  |  |  |
| *H. pylori* infection | 512 | 69.0 | 296 | 60.7 | 360 | 81.1 | 1168 | 69.8 |
| NAG |  |  |  |  |  |  |  |  |
| None | 700 | 94.3 | 470 | 96.3 | 388 | 87.4 | 1558 | 93.1 |
| Mild | 23 | 3.1 | 10 | 2.1 | 35 | 7.9 | 68 | 4.1 |
| Moderate | 16 | 2.2 | 6 | 1.2 | 17 | 3.8 | 39 | 2.3 |
| Severe | 3 | 0.4 | 2 | 0.4 | 4 | 0.9 | 9 | 0.5 |
| AG |  |  |  |  |  |  |  |  |
| None | 722 | 97.3 | 438 | 89.8 | 408 | 91.9 | 1568 | 93.7 |
| Mild | 13 | 1.8 | 33 | 6.8 | 23 | 5.1 | 69 | 4.1 |
| Moderate | 6 | 0.8 | 14 | 2.8 | 10 | 2.3 | 30 | 1.8 |
| Severe | 1 | 0.1 | 3 | 0.6 | 3 | 0.7 | 7 | 0.4 |
| IM |  |  |  |  |  |  |  |  |
| None | 612 | 82.5 | 328 | 67.2 | 368 | 82.9 | 1308 | 78.1 |
| Mild | 87 | 11.7 | 104 | 21.3 | 48 | 10.9 | 239 | 14.3 |
| Moderate | 34 | 4.6 | 45 | 9.2 | 23 | 5.1 | 102 | 6.1 |
| Severe | 9 | 1.2 | 11 | 2.3 | 5 | 1.1 | 25 | 1.5 |

Data presented as (mean ± standard deviation).BMI: Body mass index; *H. pylori*: *Helicobacter pylori*; NAG: Non-atrophic gastritis; AG: Atrophic gastritis; IM: Intestinal metaplasia.

**Table 3** **Association between risk factors and gastric precancerous lesions**

|  |  |  |  |
| --- | --- | --- | --- |
| **Risk factors** | **NAG** | **AG** | **IM** |
| **OR (95%CI)** | ***P* value** | **OR (95%CI)** | ***P* value** | **OR (95%CI)** | ***P* value** |
| Age | 1.09 (0.99-1.03) | 0.315 | 0.90 (0.88-1.01) | 0.412 | 1.01 (0.99-1.02) | 0.313 |
| BMI | 1.01 (0.93-1.11) | 0.421 | 0.89 (0.78-1.32) | 0.587 | 1.11 (0.98-1.24) | 0.134 |
| Gender | Female | - |  | - |  | - |  |
| Male | 1.52 (0.79-2.14) | 0.156 | 0.91 (0.42-1.83) | 0.792 | 1.30 (0.88-1.91) | 0.199 |
| High salt | No | - |  | - |  | - |  |
| Yes | 2.58 (1.21-4.88) | 0.011 | 0.59 (0.24-1.77) | 0.315 | 1.01 (0.55-1.41) | 0.699 |
| *H. pylori* infection | No | - |  | - |  | - |  |
| Yes | 0.59 (0.37-1.08) | 0.141 | 0.39 (0.65-0.99) | 0.041 | 0.90 (0.77-1.31) | 0.555 |
| Smoking | No | - |  | - |  | - |  |
| Yes | 2.15 (1.19-4.44) | 0.015 | 1.11 (0.77-2.10) | 0.515 | 1.97 (1.40-2.58) | 0.005 |
| Drinking | No | - |  | - |  | - |  |
| Yes | 1.00 (0.99-1.39) | 0.057 | 0.731 (0.49-1.19) | 0.161 | 0.95 (0.89-1.33) | 0.668 |
| Region | South | - |  | - |  | - |  |
| Middle | 0.33 (0.28-0.51) | 0.009 | 0.71 (0.46-1.99) | 0.669 | 2.95 (1.45-4.33) | 0.007 |
| North | 0.55 (0.40-1.01) | 0.053 | 0.33 (0.21-0.83) | 0.010 | 1.41 (0.89-2.01) | 0.313 |

BMI: Body mass index; *H. pylori*: Helicobacter pylori; NAG: Non-atrophic gastritis; AG: Atrophic gastritis; IM: Intestinal metaplasia.

**Table 4** **Comparison of the genotype distribution of the *IL-21* gene** **polymorphisms in gastric precancerous lesions**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SNPs set** | **Genotype** | **Control (*n* = 290, %)** | **NAG (*n* = 116, %)** | **AG (*n* = 106, %)** | **IM (*n* = 366, %)** | **OR1** | ***P*1 value** | **OR2** | ***P*2 value** | **OR3** | ***P*3 value** | **OR4** | ***P*4 value** | **OR5** | ***P*5 value** |
| rs907715 | TT | 70 (24.1) | 25 (21.6) | 19 (17.9) | 63 (17.2) | - |  | - |  | - |  | - |  | - |  |
| CT | 143 (49.3) | 53 (45.7) | 50 (47.2) | 170 (46.5) | 1.04 (0.60-1.81) | 0.896 | 1.29 (0.71-2.35) | 0.408 | 1.32 (0.88-1.98) | 0.179 | 1.25 (0.87-1.80) | 0.230 | 1.13 (0.62-1.74) | 0.439 |
| CC | 77 (26.6) | 38 (32.7) | 37 (34.9) | 133 (36.3) | 1.38 (0.76-2.52) | 0.290 | 1.77 (0.93-3.36) | 0.079 | 1.92 (1.24-2.98) | 0.004 | 1.77 (1.19-2.63) | 0.005 | 1.59 (1.06-2.38) | 0.013 |
| C | 220 (75.9) | 91 (78.4) | 87 (82.1) | 303 (82.8) | 1.16 (0.69-1.94) | 0.578 | 1.46 (0.83-2.56) | 0.190 | 1.53 (1.04-2.24) | 0.028 | 1.43 (1.02-2.01) | 0.039 | 1.28 (1.01-2.22) | 0.044 |
| rs12508721 | TT | 39 (13.4) | 14 (12.1) | 18 (17.0) | 54 (14.8) | - |  | - |  | - |  | - |  | - |  |
| CT | 151 (52.1) | 57 (49.1) | 55 (51.9) | 178 (48.6) | 1.05 (0.53-2.08) | 0.885 | 0.79 (0.42-1.50) | 0.467 | 0.85 (0.54-1.36) | 0.498 | 0.87 (0.57-1.33) | 0.525 | 0.82 (0.55-1.28) | 0.618 |
| CC | 100 (34.5) | 45 (38.8) | 33 (31.1) | 134 (36.6) | 1.25 (0.62-2.54) | 0.529 | 0.72 (0.36-1.42) | 0.335 | 0.97 (0.60-1.57) | 0.895 | 0.96 (0.62-1.50) | 0.863 | 0.88 (0.59-1.33) | 0.879 |
| C | 251 (86.6) | 102 (87.9) | 88 (83.0) | 312 (85.2) | 1.13 (0.59-2.17) | 0.709 | 0.76 (0.41-1.40) | 0.375 | 0.90 (0.58-1.40) | 0.634 | 0.91 (0.60-1.36) | 0.639 | 0.83 (0.49-1.19) | 0.801 |
| rs2221903 | TT | 231 (79.7) | 90 (77.6) | 83 (78.3) | 271 (74.0) | - |  | - |  | - |  | - |  | - |  |
| CT | 52 (17.9) | 24 (20.7) | 21 (19.8) | 80 (21.9) | 1.19 (0.69-2.04) | 0.539 | 1.12 (0.64-1.98) | 0.685 | 1.31 (0.89-1.94) | 0.173 | 1.25 (0.87-1.79) | 0.223 | 1.08 (0.53-1.55) | 0.459 |
| CC | 7 (2.4) | 2 (1.7) | 2 (1.9) | 15 (4.1) | 0.73 (0.15-3.60) | 0.701 | 0.80 (0.16-3.91) | 0.777 | 1.83 (0.73-4.56) | 0.190 | 1.41 (0.59-3.41) | 0.441 | 1.19 (0.41-2.87) | 0.503 |
| C | 59 (20.3) | 26 (22.4) | 23 (21.7) | 95 (26.0) | 1.13 (0.67-1.91) | 0.643 | 1.09 (0.63-1.87) | 0.769 | 1.37 (0.95-1.99) | 0.092 | 1.27 (0.90-1.79) | 0.171 | 1.08 (0.81-1.48) | 0.311 |

OR1: NAG cases compared with controls; OR2: AG cases compared with controls; OR3: IM cases compared with controls; OR4: Total gastric precancerous lesions cases compared with controls; OR5: Adjusted by age, gender, BMI, drinking status, smoking status, daily salt intake and region. SNPs: Single nucleotide polymorphisms; NAG: Non-atrophic gastritis; AG: Atrophic gastritis; IM: Intestinal metaplasia.

**Table 5 Haplotype analysis of polymorphisms in patients and controls**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **SNPs** | **Haplotype** | **Control (*n* = 290, %)** | **NAG (*n* = 116, %)** | **AG (*n* = 106, %)** | **IM (*n* = 366, %)** | ***χ*2-1** | ***P*1 value** | **OR1** | ***χ*2-2** | ***P*2 value** | **OR2** | ***χ*2-3** | ***P*3 value** | **OR3** |
| rs907715| rs12508721 | CC  | 0.496 | 0.543 | 0.575 | 0.571 | 0.713 | 0.397 | 1.20 (0.78-1.86) | 1.936 | 0.164 | 1.37 (0.88-2.15) | 3.612 | 0.057 | 1.35 (1.00-1.84) |
| CT | 0.066 | 0.060 | 0.057 | 0.071 | 0.037 | 0.847 | 1.09 (0.45-2.67) | 0.104 | 0.747 | 1.17 (0.45-3.01) | 0.077 | 0.781 | 0.92 (0.50-1.69) |
| TC | 0.163 | 0.138 | 0.047 | 0.087 | 0.368 | 0.544 | 1.21 (0.66-2.23) | 8.984 | 0.003 | 3.91 (1.51-10.11) | 8.509 | 0.004 | 2.02 (1.25-3.26) |
| TT | 0.275 | 0.259 | 0.321 | 0.271 | 0.125 | 0.724 | 1.09 (0.67-1.78) | 0.763 | 0.382 | 0.81 (0.50-1.31) | 0.024 | 0.878 | 1.03 (0.73-1.45) |
| rs2221903| rs907715 | CC  | 0.128 | 0129 | 0.123 | 0.158 | 0.002 | 0.963 | 0.99 (0.52-1.87) | 0.017 | 0.896 | 1.05 (0.53-2.06) | 1.264 | 0.264 | 0.78 (0.50-1.21) |
| CT | 0.006 | 0.026 | 0.019 | 0.014 | 2.450 | 0.118 | 0.26 (0.04-1.59) | 1.113 | 0.292 | 0.36 (0.05-2.60) | 0.701 | 0.404 | 0.50 (0.10-2.60) |
| TC | 0.434 | 0.474 | 0.509 | 0.484 | 0.527 | 0.468 | 0.85 (0.55-1.31) | 1.759 | 0.185 | 0.74 (0.47-1.16) | 1.571 | 0.210 | 0.82 (0.60-1.12) |
| TT | 0.432 | 0.371 | 0.349 | 0.344 | 1.244 | 0.265 | 1.29 (0.83-2.00) | 2.158 | 0.142 | 1.41 (0.89-2.24) | 5.157 | 0.023 | 1.44 (1.05-1.98) |

Haplotype of rs907715(C/T), rs2221903 (T/C) and rs12508721(C/T). OR1: NAG cases compared with controls; OR2: AG cases compared with controls; OR3: IM cases compared with controls. SNPs: Single nucleotide polymorphisms; NAG: Non-atrophic gastritis; AG: Atrophic gastritis; IM: Intestinal metaplasia.