

RESEARCH ARTICLE

Variants of Interleukin-16 Associated with Gastric Cancer Risk

Tao Zhang*, Hui Wang

Abstract

Aim: We conducted a case-control matched study to investigate the role of IL-16 gene polymorphisms, rs4072111, rs1131445, rs4778889 and rs11556218, in the risk of gastric cancer in a Chinese population, also performing subgroup analysis by subsites. **Methods:** To test the hypothesis of involvement, we analyzed the four SNPs of IL16 in 347 cancer patients and 368 controls. Demographic data and other information were collected using a newly designed questionnaire. Genotyping of IL16 (rs4072111, rs1131445, rs4778889 and rs11556218) was performed in a 384-well plate format on the MassARRAY® platform. **Results:** In our study, we found the gastric cancer patients were more likely to be male and have a family history of cancer ($P < 0.05$). We found the rs4778889 CC and rs11556218 GG genotype was significantly associated with 1.97 and 1.84-fold increased risk of non-cardia gastric cancer, while we did not find significant association between the four IL-16 SNPs and cardia gastric cancer. **Conclusions:** In conclusion, our study indicated that IL-16 rs4778889 CC and rs11556218 GG genotypes are associated with an increased risk of non-cardia gastric cancer in a Chinese population. Our results offer insights into the influence of IL-16 on development of gastric cancer.

Keywords: Interleukin-16 - gastric cancer - polymorphisms - Chinese population

Asian Pac J Cancer Prev, 14 (9), 5269-5273

Introduction

Worldwide, gastric cancer is the second leading cause of death from cancer, with an estimated one million new cases in 2008 (988 000 cases), accounting for 8% of all cancer-related death worldwide. More than 70% of all gastric cancer cases occurred in developing countries, and approximately half of all cases occur in China (IARC, 2008). Although many epidemiologic studies suggest that *Helicobacter pylori* (*H.pylori*) infection is one of the most important risk factors for gastric cancer, it is estimated almost 50% of the world's population are infected with *H.pylori*, but only about 1% of them occur gastric cancer (Graham et al., 1991; Parsonnet et al., 1997). Therefore, changes in lifestyle/environmental factors and improved health care as well as genetic factors may influence the susceptibility to gastric cancer (Ghoshal et al., 2007; Ghoshal et al., 2008).

The interleukin (IL) represent a diverse constellation of cytokines which regulate the function of immune system in human. They are produced predominantly by T cells, monocytes, macrophages, and endothelial cells. They have multiple functions including facilitating communication between immune cells, controlling genes, regulating transcription factors, and governing the inflammation, differentiation, proliferation, and secretion of antibodies (Salazar-Onfray et al., 2007), and the single nucleotide polymorphisms of genes encoding ILs and their receptor may alter cytokine function and dysregulate its expression, as well as cause defects in cytokine cascades (Yuzhalin, 2011). Consequently, individual genetic differences

caused by SNPs may be closely related to these disruptions and eventually play a role in gastric carcinogenesis. IL-16 is considered a proinflammatory cytokine and located on chromosome 15q26.3 in humans. IL-16 is precursor protein consisting of 631 amino acids, which is cleaved by caspase-3 to form the active C-terminal domain containing 121 amino acids (Baier et al., 1997; Drwinga et al., 1993; Zhang et al., 1998), and can promote the secretion of tumor-associated inflammatory cytokines by monocytes, such as IL-1b, IL-6 and IL-15 (Mathy et al., 2000), and play an important role in the carcinogenesis of human cancers (Schneider et al., 2000; Chung and Chang, 2003; Kai et al., 2005; Shanmugham et al., 2006). Recent experimental and epidemiological studies have demonstrated that IL-16 could be a candidate susceptibility gene in gliomas and prostate cancer (Liebrich et al., 2007; Thomas et al., 2008). Higher serum levels of IL-16 have also been associated with advanced stages of cancer and a worse patient outcome depending on the type of tumor.

Most of the studies on IL-16 gene polymorphisms were focused on the inflammatory related diseases (Gu et al., 2008; Mahindra et al., 2012; Huang et al., 2013; Milke et al., 2013), and few of them on the development of gastric cancer. Therefore, the role of IL-16 gene polymorphisms on the risk of gastric cancer was still unknown. We conducted a case-control matched study to investigate the role of IL-16 gene polymorphisms, rs4072111, rs1131445, rs4778889 and rs11556218, on the risk of gastric cancer in a Chinese population, and conducted subgroup analysis by subsites (cardia or non cardia gastric cancer).

Department of Gastrointestinal Surgery, the Centre Hospital of Wuhan, Wuhan, China *For correspondence: zhangtao61299@163.com

Table 1. Primers of Four SNPs of IL16 Genes

Variables	Primer sequence (5'-3')	Annealing temperature (°C)	Product size(bp)
rs4072111	F: CACTGTGATCCCGGTCCAGTC R: TTCAGGTACAAACCCAGCCAGC	67.3	C:164 T: 140+24
rs1131445	F: TTGATGTTGGCTGGGAAC R: CACGCTTTGAGCTTGGTG	60	T:300+160 C: 460
rs4778889	F:CTCCACACTCAAAGCCTTTTGTTCCTATGA R:CCATGTCAAACGGTAGCCTCAAGC	63	T: 246+34 T: 280
rs11556218	F: GCTCAGGTTACAGAGTGTTCATA R: TGTGACAATCACAGCTTGCCTG	60	T: 147+24 G: 171

Table 2. Distributions of Demographic and Clinic Characteristics

Characteristic	Cases N=347(%)	%	Controls N=368(%)	%	t or χ^2	P
Age, yr (Mean±SD)	56.9±7.2		57.4±8.1		0.19	0.19
Sex						
Male	216	62.3	196	53.3		
Female	131	37.7	172	46.7	5.91	0.02
Family history of cancer						
Yes	22	6.3	1	0.3		
No	325	93.7	367	99.7	21.12	<0.001
Smoking status						
Ever	98	28.2	87	23.6		
Never	249	71.8	281	76.4	1.97	0.16
Drinking status						
Ever	116	33.4	120	32.6		
Never	231	66.6	248	67.4	0.05	0.82
Subsites of gastric cancer						
Non-cardia	228	65.7				
Cardia	119	34.3				
Stage of gastric cancer						
Early gastric cancer	134	38.6				
Advanced gastric cancer	213	61.4				

Materials and Methods

Study population and design

All the subjects were collected from the Centre Hospital of Wuhan between December 2008 and November 2011. A total of 385 patients with newly histopathologically confirmed primary gastric cancer, including cardia and non-cardia gastric cancer, were included in our study. Of 385 patients, 347 patients were willing to participate into our study, with a participation rate of 90.1%. All the cases were selected from the Centre Hospital of Wuhan. Patient who suffered from secondary or recurrent tumors were excluded from our study. A total of 426 controls were selected from the same hospital during the same time period from outpatients in Surgical Department, Plastic Surgery Department and ENT Department. Finally, 368 controls agreed to participate into our study, with a participation rate of 86.4%. All patients were asked to provide 5ml blood samples for DNA extraction.

Genotyping

Blood samples, collected and stored as described above, were collected from all study participants in EDTA-coated tubes. The buffy coat was collected and total DNA was extracted using a TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). We selected potential functional SNPs of interested XPF from Database of single nucleotide polymorphisms (SNPs) of NCBI(<http://www.ncbi.nlm.nih.gov/>) and SNPinfo (<http://snpinfonihs.nih.gov/>) with the following criteria: (1) the minor allele frequency $\geq 10\%$ of the Chinese population; (2) influencing the microRNA binding sites activity.

Genotyping of IL16 (rs4072111, rs1131445, rs4778889 and rs11556218) was performed in a 384-well plate format on the MassARRAY® platform (Sequenom®, San Diego, CA, USA), which combines polymerase chain reaction (PCR) and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry technologies. PCR single base extension (SBE) primers (Table 1) were designed using Sequenom® Assay Design, Version 3.1 software (Sequenom®), according to the manufacturer's instructions. Each PCR reaction mix comprised 50 ng genomic DNA, 200 μ M dNTP, 2.5 U Taq DNA polymerase (Promega, Madison, WI, USA) and 200 μ M primers, in a total volume of 20 μ l. The cycling programme involved preliminary denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 64°C for 30 s, and extension at 72°C for 1 min. PCR products were verified by 1.0% agarose gel electrophoresis and visualized via ethidium bromide staining and ultraviolet light. Genotyping was performed without knowledge of the case/control status of the subjects, and reproducibility was confirmed by repeat analysis of a randomly chosen subgroup of 5% of study participants.

Statistical analysis

Continuous variables were presented as mean \pm SD and analysed using the independent-samples t-test. Categorical variables were presented as n (%) of subjects and analysed using the χ^2 -test. The Hardy-Weinberg equilibrium and between-group comparison of genotype distribution were analyzed using the χ^2 -test. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were used to assess the effect of each SNP on CAD risk. Multivariate logistic regression analysis was performed to calculate the OR (95% CI) after adjusting for sex, smoking status, BMI, hypertension, diabetes, TC, TG, LDL-C and HDL-C. A P -value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS® software, version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®.

Statistical analysis

Results

The demographic and clinic characteristics of the selected cases and controls were shown in Table 2. The mean ages of the 347 cases with gastric cancer and 368

Table 3. IL-16 Genotypes and Alleles with Overall Non-cardia and Cardia Gastric Cancer Risk

Variables	Controls(%)	Non-cardia(%)	OR(95% CI) ¹	P value	Cardia(%)	OR(95% CI) ¹	P value
rs4072111							
CC	251(72.4)	158(69.4)	1	-	84(70.5)	1	-
CT	54(15.6)	37(16.2)	1.09(0.67-1.77)	0.72	19(16.1)	1.05(0.56-1.92)	0.87
TT	42(12)	33(14.4)	1.25(0.73-2.11)	0.38	16(13.4)	1.14(0.57-2.20)	0.69
T allele	96(27.7)	70(30.6)	1.16(0.79-1.70)	0.43	35(29.5)	1.09(0.67-1.76)	0.71
C allele	305(87.9)	195(85.6)	1.02(0.77-1.34)	0.91	103(86.6)	1.01(0.71-1.43)	0.96
rs1131445							
TT	174(50.2)	108(47.4)	1	-	57(47.9)	1	-
TC	112(32.4)	75(32.9)	1.08(0.73-1.60)	0.69	39(32.9)	1.06(0.64-1.75)	0.8
CC	60(17.4)	45(19.7)	1.21(0.75-1.95)	0.41	23(19.3)	0.97(0.51-1.82)	0.92
C allele	172(49.6)	120(52.6)	1.12(0.79-1.59)	0.49	62(52.1)	1.10(0.71-1.71)	0.65
T allele	286(82.4)	183(80.3)	1.03(0.75-1.41)	0.84	96(80.7)	1.01(0.68-1.51)	0.94
rs4778889							
TT	212(61.2)	119	1	-	69(57.8)	1	-
TC	106(30.5)	77	1.29(0.87-1.90)	0.17	37(31.4)	1.07(0.65-1.74)	0.77
CC	29(8.3)	32	1.97(1.09-3.54)	0.01	13(10.8)	1.38(0.62-2.91)	0.37
C allele	135(38.9)	109(47.8)	1.44(1.01-2.05)	0.03	50(42.2)	1.14(0.73-1.77)	0.55
T allele	318(91.6)	196(85.9)	1.10(0.82-1.48)	0.52	106(89.2)	1.02(0.71-1.48)	0.89
rs11556218							
TT	265(76.4)	160(70.2)	1	-	87(73.2)	1	-
TG	55(15.8)	38(16.7)	1.14(0.70-1.85)	0.56	20(16.7)	0.96(0.51-1.83)	0.92
GG	27(7.8)	30(13.1)	1.84(1.02-3.34)	0.03	12(10.1)	1.35(0.60-2.90)	0.41
T allele	82(23.6)	68(29.8)	1.37(0.92-2.04)	0.09	32(26.8)	1.19(0.71-1.95)	0.48
T allele	320(92.2)	198(86.9)	1.03(0.78-1.35)	0.86	107(89.9)	1.02(0.73-1.43)	0.91

¹Adjusted for sex, age and family history of cancer

controls were 56.9±7.2 and 57.4±8.1 years, respectively. We did not find significant differences in the ages distributions between cases and controls ($P > 0.05$). There was no significant difference in the drinking and smoking status between the two groups. However, the gastric cancer patients were more likely to be male and have a family history of cancer ($P < 0.05$). Of the cancer cases, 65.7% were non-cardia and 34.3% was at early gastric cancer.

Analyses of genotype and allele frequencies of IL16 rs4072111, rs1131445, rs4778889 and rs11556218 were stratified by subsites of gastric cancer among gastric cancer and control groups, and showed in Table 3. We observed the rs4778889 CC genotype was significantly associated with increased risk of non-cardia gastric cancer (OR=1.97, 95%CI=1.09-3.54), and T allele of rs4778889 was observed a significant high risk of non-cardia gastric cancer (OR=1.44, 95% CI=1.01-2.05). Similarly, we found GG genotype of rs11556218 showed a 1.84-fold increased risk of non-cardia gastric cancer (OR=1.84, 95% CI=1.02-3.34). For cardia gastric cancer, we did not find a significant association between the four SNPs and cancer risk.

Discussion

The IL-16 generally functions as an immunosuppressor and anti-inflammatory mediator, and has been implicated in autoimmune disease and progression of malignancies (Azimzadeh et al., 2012; Yellapa et al., 2012; Li et al., 2011; Gao et al., 2009a). Currently, there was only one study reported the association between IL-16 polymorphisms and risk of gastric cancer (Gao et al., 2009a), and this case-control study indicated that rs11556218T/G and rs4072111C/T polymorphisms of the IL-16 gene was significantly associated with the susceptibility to gastric

cancer patients. In our case-control study, we analyzed genetic polymorphisms of IL-16 rs4072111, rs1131445, rs4778889 and rs11556218 for gastric cancer risk in a Chinese population. The main finding in our study was that IL-16 rs4778889 CC and rs11556218 GG genotypes were associated with an increased risk of developing non-cardia gastric cancer, while IL-16 rs4072111C>T and rs1131445T>C polymorphisms had no association. Moreover, we did not find the four IL-16 SNPs were association with risk of cardia gastric cancer.

The rs11556218T/G polymorphism is located in the exon 6 region of the IL-16 gene, this is a missense mutation, wherein asparagine(Asn) is substituted by lysine(Lys). Recently, several studies reported that rs11556218T/G polymorphisms were associated with risk of various diseases (Gao et al., 2009a; Azimzadeh et al., 2011; Wu et al., 2011; Batai et al., 2012). A recent study conducted in Sichuan of China reported that rs11556218T/G polymorphism was significantly associated with the susceptibility to NPC, and TG genotype was associated with a significantly higher risk of NPC as compared with the TT genotype (Gao et al., 2009a). Another study also conducted in China showed that the TG/GG genotypes of rs11556218T/G were associated with a significantly increased risk of coronary artery disease as compared with the TT genotype, with a odds ratio (95% CI) of 1.77(1.16-2.71) (Wu et al., 2011). In our study, we found that rs11556218T/G polymorphism was associated with a statistically significant association with gastric cancer, which was in line with previous studies. Moreover, Gao et al. showed the G allele in controls from the Chinese population was 24.3%, while that in the patients with cancer disease was 32% (Gao et al., 2009a). In our study, the proportion of G allele in the controls was 23.6%, which was similar to that reported by Gao et al. (2009a).

The rs4778889C/T polymorphism is located at 295 bp upstream from the start site of transcription and is associated with altered levels of gene expression (Nakayama et al., 2000). Compared with rs11556218T/G, evidences of association of rs4778889C/T polymorphism with disease are limited. Only three studies assessed the association between rs4778889C/T polymorphism and risk of disease (Gao et al., 2009a; Gao et al., 2009b; Azimzadeh et al., 2011). Azimzadeh et al. (2011) reported that IL-16 rs4778889C/T polymorphism showed significant association with 0.192 fold decreased risk of colorectal cancer. However, another two studies conducted in China reported the IL-16 rs4778889C/T polymorphism has no role in the development of gastric cancer, colorectal cancer and nasopharyngeal cancer (Gao et al., 2009a; Gao et al., 2009b). Our study reported an increased risk of gastric cancer, which was not in line with previous studies. The inconsistency of these studies may be explained by differences in ethnicities, source of control subjects, sample size and etc. Further their confirmation of existing findings is still needed in future studies.

In the sub-analysis, we found that IL-16 rs4778889 CC and rs11556218 GG genotypes were associated with increased risk of non-cardia gastric cancer, but no association with cardia gastric cancer. This inconsistency between the non-cardia and cardia gastric cancer results could be induced by the etiology, pathology, carcinogenesis, and prognosis of cardia and non-cardia gastric cancer. This possibility is also indirectly supported by previous studies (Ni et al., 2012; Xue et al., 2012), which indicated a lack of association of Interleukin promoter polymorphisms with risk of non-cardia cancer. Caution should be taken when interpreting the significance of these findings, because the sample size of non-cardia gastric cancer cases and controls in our study are relatively same, and they may not represent the same population. Therefore, further large sample size study with a priori hypothesis for cardia and non-cardia gastric cancer risk is warranted.

In conclusion, our study indicated that IL-16 rs4778889 CC and rs11556218 GG genotypes are associated with an increased risk of non-cardia gastric cancer in a Chinese population, but no significant association was found in cardia gastric cancer. Our results should be confirmed in future large sample size study. Because the polymorphism of IL-16 can increase the risk of cancer, it could be used to explore the role of IL-10 polymorphisms in the development of gastric cancer in different clinical stages and different subsites.

Acknowledgements

We thank all participating subjects for their cooperation in this study, and thank for doctors and nurses who helped recruit all the subjects in our study.

References

- Azimzadeh P, Romani S, Mohebbi SR, et al (2012). Association of polymorphisms in microRNA-binding sites and colorectal cancer in an Iranian population. *Cancer Genet*, **205**, 501-7.
- Baier M, Bannert N, Werner A, et al (1997). Molecular cloning, sequence, expression, and processing of the interleukin 16 precursor. *Proc Natl Acad Sci U S A*, **94**, 5273-7.
- Batai K, Shah E, Murphy AB, et al (2012). Fine-mapping of IL16 gene and prostate cancer risk in African Americans. *Cancer Epidemiol Biomarkers Prev*, **21**, 2059-68.
- Chung YC, Chang YF (2003). Serum interleukin-6 levels reflect the disease status of colorectal cancer. *J Surg Oncol*, **83**, 222-6.
- Drwanga HL, Toji LH, Kim CH, et al (1993). NIGMS human/rodent somatic cell hybrid mapping panels 1 and 2. *Genomics*, **16**, 311-4.
- Gao LB, Liang WB, Xue H, et al (2009b). Genetic polymorphism of interleukin-16 and risk of nasopharyngeal carcinoma. *Clin Chim Acta*, **409**, 132-5.
- Gao LB, Rao L, Wang YY, et al (2009a). The association of interleukin-16 polymorphisms with IL-16 serum levels and risk of colorectal and gastric cancer. *Carcinogenesis*, **30**, 295-9.
- Ghoshal UC, Tiwari S, Dhingra S, et al (2008). Frequency of Helicobacter pylori and CagA antibody in patients with gastric neoplasm and controls: the Indian enigma. *Dig Dis Sci*, **53**, 1215-22.
- Ghoshal UC, Tripathi S, Ghoshal U (2007). The Indian enigma of frequent H. pylori infection but infrequent gastric cancer: is the magic key in Indian diet, host's genetic make up, or friendly bug? *Am J Gastroenterol*, **102**, 2113-4.
- Graham DY, Adam E, Reddy GT, et al (1991). Seroepidemiology of Helicobacter pylori infection in India; comparison of developing and developing countries. *Dig Dis Sci*, **36**, 1084-8.
- Gu XJ, Cui B, Zhao ZF, et al (2008). Association of the interleukin (IL)-16 gene polymorphisms with Graves' disease. *Clin Immunol*, **127**, 298-302.
- Huang H, Zeng Z, Zhang L, et al (2013). The association of interleukin-16 gene polymorphisms with susceptibility of coronary artery disease. *Clin Biochem*, **46**, 241-4.
- International Agency for Research on Cancer (2008). Globocan 2008: Stomach Cancer incidence, Mortality and Prevalence Worldwide in 2008. IARC.
- Kai H, Kitadai Y, Kodama M, et al (2005). Involvement of proinflammatory cytokines IL-1beta and IL-6 in progression of human gastric carcinoma. *Anticancer Res*, **25**, 709-13.
- Liebrich M, Guo LH, Schluesener HJ, et al (2007). Expression of interleukin-16 by tumor-associated macrophages/activated microglia in high-grade astrocytic brain tumors. *Arch Immunol Ther Exp (Warsz)*, **55**, 41-7.
- Li S, Deng Y, Chen ZP, et al (2011). Genetic polymorphism of interleukin-16 influences susceptibility to HBV-related hepatocellular carcinoma in a Chinese population. *Infect Genet Evol*, **11**, 2083-8.
- Mahindra A, Anderson KC (2012). Role of interleukin 16 in multiple myeloma pathogenesis: a potential novel therapeutic target? *J Natl Cancer Inst*, **104**, 964-5.
- Mathy NL, Scheuer W, Lanzendörfer M, et al (2000). Interleukin-16 stimulates the expression and production of pro-inflammatory cytokines by human monocytes. *Immunology*, **100**, 63-9.
- Milke L, Schulz K, Weigert A, et al (2013). Depletion of tristetraprolin in breast cancer cells increases interleukin-16 expression and promotes tumor infiltration with monocytes/macrophages. *Carcinogenesis*, **34**, 850-7.
- Nakayama EE, Wasi C, Ajisawa A, et al (2000). A new polymorphism in the promoter region of the human interleukin-16 (IL-16) gene. *Genes Immun*, **1**, 293-4.
- Ni P, Xu H, Xue H, et al (2012). A meta-analysis of interleukin-10-1082 promoter polymorphism associated

- with gastric cancer risk. *DNA Cell Biol*, **31**, 582-91.
- Parsonnet J, Friedman GD, Orentreich N, et al (1997). Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. *Gut*, **40**, 297-301.
- Salazar-Onfray F, López MN, Mendoza-Naranjo A (2007). Paradoxical effects of cytokines in tumor immune surveillance and tumor immune escape. *Cytokine Growth Factor Rev*, **18**, 171-82.
- Schneider MR, Hoefflich A, Fischer JR, et al (2000). Interleukin-6 stimulates clonogenic growth of primary and metastatic human colon carcinoma cells. *Cancer Lett*, **151**, 31-8.
- Shanmugham LN, Petrarca C, Frydas S, et al (2006). IL-15 an immunoregulatory and anti-cancer cytokine. Recent advances. *J Exp Clin Cancer Res*, **25**, 529-36.
- Thomas G, Jacobs KB, Yeager M, et al (2008). Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet*, **40**, 310-5.
- Wu J, Wang Y, Zhang Y, Li L (2011). Association between interleukin-16 polymorphisms and risk of coronary artery disease. *DNA Cell Biol*, **30**, 305-8.
- Xue H, Lin B, An J, et al (2012). Interleukin-10-819 promoter polymorphism in association with gastric cancer risk. *BMC Cancer*, **12**, 102.
- Yellapa A, Bahr JM, Bitterman P, et al (2012). Association of interleukin 16 with the development of ovarian tumor and tumor-associated neoangiogenesis in laying hen model of spontaneous ovarian cancer. *Int J Gynecol Cancer*, **22**, 199-207.
- Yuzhalin A (2011). The role of interleukin DNA polymorphisms in gastric cancer. *Hum Immunol*, **72**, 1128-36.
- Zhang Y, Center DM, Wu DM, et al (1998). Processing and activation of pro-interleukin-16 by caspase-3. *J Biol Chem*, **273**, 1144-9.