



Association of *IL-1B+3954* and *IL-1RN* Polymorphisms in Chronic Gastritis and Peptic Ulcer

Neda MOTAMEDI RAD¹, Meysam REZAEISHAHMIRZADI², Sepideh SHAKERI², Mohamad Reza ABBASZADEGAN³, *Mohammad SHEKARI⁴

1. Dept. of Medical Genetics, School of Medicine, Hormozgan University of Medical Sciences, Bandar-Abbas, Iran
2. Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Dept. of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4. Center for Research of Molecular Medicine, Hormozgan University of Medical Sciences, Bandar-Abbas, Iran

*Corresponding Author: Email: mshekari45@yahoo.com

(Received 04 Sep 2017; accepted 12 Dec 2017)

Abstract

Background: *Helicobacter pylori* are the main cause of chronic inflammation and peptic ulcer. We aimed to determine if *IL-1B+3954* and *IL-1RN* polymorphisms are associated with the risk of chronic gastritis and peptic ulcer in Iranian population.

Methods: In this case-control study, from 198 individuals enrolled by Mohammadi Hospital, Bandar Abbas, southern Iran from 2012 to 2014 and who showed the symptoms of chronic gastritis and 84 with peptic ulcer participated in the case group, two biopsies were taken from the body, antrum, or ulcer edge of each patient. Individuals without chronic gastritis or peptic ulcer were selected as the control group and we also confirmed the presence of anti-*H. pylori* serum IgG in 321 control subjects. *IL-1B+3954C/T* polymorphism was analyzed through PCR-RFLP, while the *IL-1RN* polymorphism was analyzed via PCR-based VNTR.

Results: *IL-1B+3954 TT* was associated with a high risk of gastritis and peptic ulcer [Odds Ratio (OR)]=2.63, 95% Confidence Interval (CI)= (1.47-4.70) (OR=3.40, CI=1.72-6.71) respectively and the *IL-1B+3954 T* allele was associated with chronic gastritis (OR=1.64, 95% CI=1.13-2.36). Moreover, patient carrying *IL-1RN L/2* and allele 2 showed an increased risk of peptic ulcer (OR=2.97, CI=1.72-5.11, OR=1.64, CI=1.13-2.36), respectively.

Conclusion: *IL-1B* and *IL-1RN* are associated with an increased risk for chronic gastritis and peptic ulcer disease.

Keywords: Interleukin, Gastritis, Peptic ulcer, Polymorphism

Introduction

Helicobacter pylori is a Gram-negative microaerophilic bacterium, whose infection is one of the major causes of chronic gastritis and peptic ulcer disease (PUD) in human beings (1, 2). A majority of the world population is reported to be infected with *H. pylori*, but only 20% of the infected individuals develop clinically significant disease (3).

While most infected individuals remain asymptomatic, persistent colonization and chronic inflammation increase the risk of developing atrophic gastritis, peptic ulcers, and gastric cancer (4, 5). The clinical outcome of *H. pylori* infection can be determined by the genetic characteristics of the host and the bacteria, and also by envi-

ronmental factors (6). Recently, inflammatory response is one essential part of the pathogenesis of PUD. Evidence also suggests that inflammatory cytokines, besides the genetic variation in the genes that encode the inflammatory mediators, might play a key role in the development of PUD (7, 8).

The interleukin-1 (*IL-1*) gene cluster on chromosome 2q contains three related genes within a 430-kb region: *IL-1A*, *IL-1B*, and *IL-1RN*, which encode the pro-inflammation cytokines *IL-1a* and *IL-1b* and the endogenous receptor antagonist *IL-1ra*, respectively (9). *IL-1b* is up-regulated in the presence of *H. pylori* and is a potent inhibitor of gastric acid secretion (10). Three di-allelic polymorphisms in *IL-1B* have been reported, all representing C-T base transitions, at positions -511, -31, and +3954 bp. *IL-1RN* has a Penta-allelic 86-bp tandem repeat polymorphism in intron-2, while *IL-1RN* allele 2 has been shown to be associated with enhanced *IL-1b* production (11). Polymorphisms have been reported to be related to variations in the production levels of *IL-1b* and *IL-1ra* (12).

In this study, we investigated whether *IL-1B* (rs1143634) and *IL-1RN* polymorphisms are associated with chronic gastritis or peptic ulcer in Iran.

Methods

We conducted a case-control study, 282 patients (including 198 patients with chronic gastritis and 84 patients with peptic ulcer) who underwent an endoscopic study in a specialized unit for Gastroenterology Endoscopy in the city of Bandar Abbas in 2012 to 2014, southern Iran were included in this study. All the study participants belonged to Hormozgan Province in Iran, and all of them suffered from functional dyspepsia and epigastric pain. These patients did not receive *H. pylori* eradication therapy. Moreover, we selected those individuals who had no signs of dyspepsia, no history of gastric diseases, and no indication for endoscopy, and used IgG-Anti *H. pylori* ELISA test to diagnose *H. pylori* infection in them. Finally,

321 people were positive for serum *H. pylori* IgG indicating their previous exposure to *H. pylori*. However, they had no signs of gastric diseases so they were selected as the control group.

In both groups, the researcher excluded the subjects under non-steroidal anti-inflammatory drug (NSAID) treatment and those with autoimmune gastritis.

Informed consent was obtained from the participants, and they were informed that the results would be used for developing better treatment strategies for such diseases. Hormozgan Bioethics Committee of the Medical University approved the validity of this study.

Details of the study participants such as diet, socio-demographic factors, family history of gastritis or ulcers, and smoking habits were recorded in surveys.

Endoscopy and Gastric Histology

Every patient underwent an endoscopy using a video processor and a video gastroscope (Fujinon, Wayne, NJ, USA). In each patient, we took two biopsies from the antrum, corpus, or ulcer edge; one specimen was immediately fixed in formalin for histological testing and the other was placed in distilled water for the diagnostic test. The biopsies collected for the diagnostic test were stored at -20°C until the processing procedures. Furthermore, the histological sections were stained with hematoxylin and eosin and evaluated by a pathologist using updated Sydney System criteria (13). Endoscopic observation and histopathological confirmation were applied to determine the patient's pathology in the study. And we finally selected 282 individuals *Helicobacter pylori* positive as patient group.

Serology

Blood samples (5 ml) were collected from all the control group subjects. The serum was tested for IgG anti-*H. pylori* by ELISA; International Immuno-Diagnostics, Foster City, CA, USA) following the manufacturer's instructions. The sensitivity and specificity of this method was 96%. A subject was considered *H. pylori* positive if we detected antibodies in the serum.

Analysis of *IL1B* and *IL1RN* Genes

In this study, genomic DNA was extracted from peripheral blood using the QIA amp DNA Mini Kit. *IL-1B*+3954C/T polymorphisms were analyzed by RFLP with the *TaqI* enzyme (14). The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with 0.1% ethidium bromide. PCR products were digested with 2 units of *TaqI* for 8 h at 65°C and resulted in an intact fragment of 249 bp (allele T) or in two fragments of 135 and 114 bp (allele C). Fragments were analyzed by electrophoresis on 3% agarose gels stained with 0.1% ethidium bromide (Fig.1).

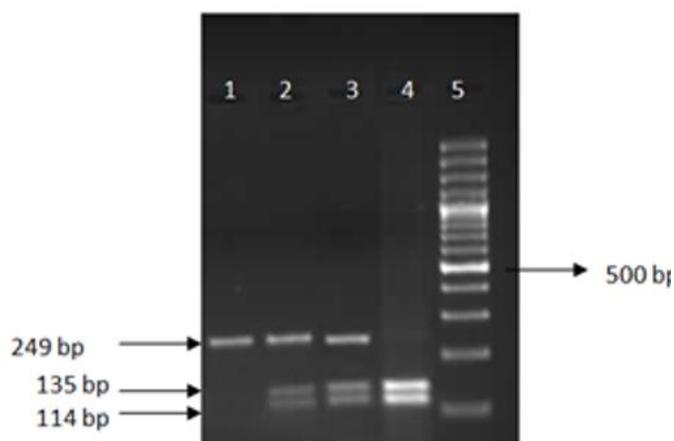


Fig. 1: Agarose gel showing interleukin (*IL*)-1*B* *TaqI*-restriction patterns in four individuals. Lane 1 homozygous for allele T(*TT*) lanes 2,3 show an individual heterozygous (*CT*), and lane 4 homozygous for allele C(*CC*), A 100 basepair ladder is shown in the right lane (5)

IL-1RN Gene Polymorphism

The Penta-allelic variable number of 86-bp VNTR in intron 2 of the *IL-1RN* gene was analyzed by PCR. The oligonucleotides 5' CTCAGCAACACTCCTAT 3' and 5' TCCTGGTCTGCAGGTAA 3' were used as primers (12). The PCR products resulting in a short allele with two repeats of the 86-bp (*IL-1RN**2) or long alleles (*IL-1RN**L): allele 1 (four repeats), allele 3 (five repeats), allele 4 (three repeats), and allele 5 (six repeats) were analyzed by electrophoresis on 2% agarose gel stained with 0.1% ethidium bromide (Fig.2).

Statistical analysis

Hardy-Weinberg equilibrium (HWE) of allelic frequencies at individual loci was assessed by comparing the observed and expected genotype frequencies by incorporating the χ^2 test (for *IL-1B* $P=0.197$; for *IL-1RN* $P=1.00$). For statistical analysis, the χ^2 test was used to assess the statistical significance of the differences between the genotype and allelic frequencies of the population. Odds ratios (OR) with a 95% confidence interval (CI) were calculated for genotyping objectives. The level of significance was set at $P<0.05$.

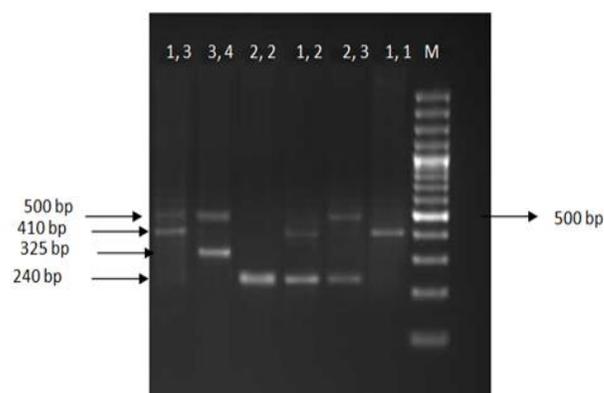


Fig. 2: Agarose gel showing six different patterns for interleukin (*IL*)-*1RN* genotypes 1,1. 2,2. 1,2. 1,3. 2,3. And 3,4. A base pair ladder is shown in the right lane (M)

Results

The case group represented 198 individuals with chronic gastritis and 84 patients with peptic ulcer in this study. The average age of the patients with gastritis was 41 yr with an age range of 17 to 85 yr; the peptic ulcer patients had an average age of 42 yr, ranging from 23 to 72 yr. Subjects in the control group had an average age of 41 yr and their age ranged from 21 to 74 yr. There were significant differences among the three groups in family history of ulcer and smoking habits (Table 1).

The relationships between individual *IL-1B* and *IL-1RN* genotypes among patients with a risk of chronic gastritis and peptic ulcer are represented in Tables 2 and 3.

Table 1: Demographic Characteristics of Cases and Controls

	Control (321) n (%)	Gastritis (198) n (%)	P	Peptic ulcer (84) n (%)	P
Age (yr)	41.8 ±15.9	41.2±16.1	0.96	42.8 ±15.5	0.31
Sex			0.79		0.73
Male	162 (50.4)	103 (52)		40 (47.6)	
Female	159 (49.6)	95 (48)		44(52.4)	
Smoking habit			<0.001		<0.001
Smoker	51 (15.9)	67 (33.8)		34 (40.5)	
Nonsmoker	270 (84.1)	131 (66.2)		50 (59.5)	
Family history ulcer			<0.001		<0.001
Yes	46 (14.3)	66 (33.3)		34 (40.4)	
No	275 (85.7)	123 (66.7)		50 (59.6)	

The genotype frequencies of the *IL-1B* +3954 *TC* and *TT* were significantly different between gastritis and control group (OR=1.40, CI=0.94-2.09, and OR=2.63, CI=1.47-4.70) respectively. Moreover, the allele frequency of *IL-1B* +3954 *T*

was significantly different between gastritis and control (OR=1.64, CI=1.13-2.36) but there were no significant differences in any allele or genotype distribution of *IL-1RN* polymorphism between gastritis and control group.

Table 2: Genotype and allele frequencies of *IL-1B* and *IL-1RN* polymorphisms in gastritis and control

Polymorphism	genotype	Gastritis (198) n (%)	Control (321) n (%)	OR^a(95%CI)	P^a
<i>IL-1B</i>	<i>CC</i>	84 (42.4)	178 (55.5)	1.0	-
	<i>TC</i>	79 (39.9)	116 (36.1)	1.40(0.94-2.09)	0.08
	<i>TT</i>	35 (17.7)	27 (8.4)	2.63(1.47-4.70)	<0.001
	<i>TC+TT</i>	114 (57.6)	143(44.5)	1.64(1.13-2.36)	0.008
<i>IL-1RN</i>	<i>L/L</i>	150(75.8)	240(74.8)	1.0	-
	<i>L/2</i>	40(20.2)	63 (19.6)	0.99(0.62-1.56)	0.96
	<i>2/2</i>	8 (4)	18(5.6)	0.83(0.36-1.89)	0.66
	<i>L/2+2/2</i>	48 (24.2)	81(25.2)	0.95(0.62-1.45)	0.83

^aAdjusted for smoking and family history ulcer

Table 3: Genotype and allele frequencies of *IL-1B* and *IL-1RN* polymorphisms in peptic ulcer and control

Polymorphism	Genotype	Peptic ulcer (84)n (%)	Control (321)n (%)	OR^a(95%CI)	P^a
<i>IL-1B</i>	<i>CC</i>	42(50)	178(55.5)	1.0	-
	<i>TC</i>	19(22.6)	116(36.1)	0.62(0.33-1.16)	0.13
	<i>TT</i>	23(27.4)	27(8.4)	3.40(1.72-6.71)	<0.001
	<i>TC+TT</i>	42(50)	143(44.5)	0.95(0.62-1.45)	0.83
<i>IL-1RN</i>	<i>L/L</i>	45(53.6)	240(74.8)	1.0	-
	<i>L/2</i>	36(42.9)	63(19.6)	2.97(1.72-5.11)	<0.001
	<i>2/2</i>	3(3.5)	18(5.6)	0.87(0.22-3.20)	0.83
	<i>L/2+2/2</i>	39(46.4)	81(25.2)	1.64(1.13-2.36)	<0.001

^a Adjusted for smoking and Family history ulcer

There was significant association between the *IL-1BTT* genotype and peptic ulcer (OR=3.40, CI=1.72-6.71) but there was no significant association between *TC* genotype and *T* allele in peptic ulcer and control group. In peptic ulcer group there was significant association between *L/2* genotype and *2* alleles as compared to control group (OR=2.97, CI=1.72-5.11 and OR=1.64, CI=1.13-2.36). However, there were no differences in the frequency of the *IL-1RN2/2* genotype between peptic ulcer and control.

Discussion

In this study, homozygous variant (*TT*) of *IL-1B+3954* gene is associated with peptic ulcer and carrying *T* allele (*CT+TT*) is correlated with gastritis. Heterozygous genotype (*L/2*) and carrying *2* alleles of *IL-1RN* (*L/2+ 2/2*) an increased risk of peptic ulcer.

Different pathological mechanisms related to the progression of chronic gastritis and peptic ulcer is suggested (12). *H. pylori* infection can cause hypochlorhydria, gastric atrophy, and eventually peptic ulcer and cancer. In fact, the interaction between the microorganism and the host immune system will lead to diseases. After *H. pylori* infection, the local immune cells are activated and exhibit immune responses affecting the disease process. For example, increased production of pro-inflammatory cytokines in the mucus (such as *IL-1*, *IL-6*, *IL-10*, *TNF- α* , and interferon- γ) of those who are at risk of *H. pylori* infection is associated with gastritis induced by *H. pylori* and the severity of inflammation (15). *IL-1B* is a member of the *IL-1* family and a pro-inflammatory cytokine involved in the immune defense against infection. *IL-1B* expression increases the expression of factors adhered to vascular endothelial cells that cause the leukocytes to extravagate to the site of infection and resets the temperature regulation center in the hypothalamus which leads to increased body temperature. These are the activities of body immune system against infection. In addition, for the role of *IL-1B* in adjusting a secretion of acid and protecting cell activities in gastric mucus, there is a body of re-

search related to *IL-1B* polymorphism, peptic and gastric ulcer. Also, it is reported that *IL-1B* is a cell-protecting molecule and strongly limits the gastric acid secretion (16). Therefore, *IL-1* gene polymorphisms may be associated with the severity of inflammatory diseases, autoimmune diseases, such as rheumatoid arthritis, and cancers, including gastric cancer (17).

In the context of such subjects, the effects of polymorphism in two cytokine genes, the pro-inflammatory cytokine *IL-1B* and anti-inflammatory cytokine *IL-1RN*, in relation to the development of peptic ulcer and chronic gastritis were analyzed. In this study, a significant association was observed between *IL-1B+3954TT* and *T* allele genotype and patients with gastritis and *TT* genotype and peptic ulcer compared with the control group. Since the presence of *TT* polymorphism in *IL-1B* has been reported as associated with increased gene expression (18) and this increased expression enhances the severity of the gastric mucosal inflammatory responses (19), carriers of the *TT* genotype are susceptible to peptic ulcer and gastritis. Similar results have been obtained from the populations of Poland(20), African Americans and Caucasian (21), but this association was not found in the Chinese population (22). This difference in results can be due to different genetic backgrounds in different ethnic groups.

In addition, we conducted on *IL-1RN* polymorphism, a significant association was also observed between the genotype *L/2* and carriers of the allele *2* and peptic ulcer. *IL-1RN* is involved in gastric diseases. The allele *2* is associated with increased risk of precancerous lesions indicating the role of this polymorphism in the early stages of gastric carcinogenesis (23). In another study on the Caucasian population, the allele *2* had a significant association with increased risk of gastric cancer (24), but this association was not observed in the Korean population (11). In addition, in a study on the cytokine polymorphisms in which a population of Southern Brazil was involved, a significant association was observed between the frequency of the allele *2* and development of gastric cancer (25). These differences

may be due to lower frequency of the risk allele in some populations, such as the Asian population.

In this study, the frequencies of allele 2 and L/2 were 46.4%, 42.9% and 25.2%, 19.6% in patients with peptic ulcer and control individuals, respectively. Since the allele 2 is associated with increased production of the IL-1B, which limits the amount of gastric acid and that acid reduction is favorable for bacterial colonization, the allele 2 can play a role in the development of gastric diseases with the presence of *H. pylori* (19).

Conclusion

This study supports the previous evidence on the role of inflammatory factors in development of gastric diseases in the Caucasian population which can potentially help to identify the patients at risk and to apply new methods of treatment in the future.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

We wish to thank Dr. Malekzadeh, Ms. Abedi and Ms. Mohseni for their kind help during this research. The budget was supported by Hormozgan University of Medical Science.

Conflict of interest

The authors declare that there is no conflict of interests.

References

1. Barbosa HPM, Martins LC, dos Santos SEB, et al (2009). Interleukin-1 and TNF- α

- polymorphisms and *Helicobacter pylori* in a Brazilian Amazon population. *World J Gastroenterol*, 15 (12): 1465-71.
2. Qadri Q, Rasool R, Afroze D, et al (2014). Study of TLR4 and IL-8 Gene Polymorphisms in *H. pylori*-Induced Inflammation in Gastric Cancer in an Ethnic Kashmiri Population. *Immunol Invest*, 43 (4): 324-36.
3. Chen A, Li CN, Hsu PI, et al (2004). Risks of interleukin-1 genetic polymorphisms and *Helicobacter pylori* infection in the development of gastric cancer. *Aliment Pharmacol Ther*, 20 (2): 203-11.
4. Rad R, Prinz C, Neu B, et al (2003). Synergistic effect of *Helicobacter pylori* virulence factors and interleukin-1 polymorphisms for the development of severe histological changes in the gastric mucosa. *J Infect Dis*, 188 (2): 272-81.
5. Wang YM, Li ZX, Tang FB, et al (2016). Association of genetic polymorphisms of interleukins with gastric cancer and precancerous gastric lesions in a high-risk Chinese population. *Tumour Biol*, 37 (2): 2233-42.
6. Peek RM, Blaser MJ (2002). *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer*, 2 (1): 28-37.
7. Yin YW, Hu AM, Sun QQ, et al (2013). Association between interleukin-8 gene-251 T/A polymorphism and the risk of peptic ulcer disease: A meta-analysis. *Hum Immunol*, 74 (1): 125-30.
8. Kumar S, Kumari N, Mittal RD, et al (2015). Association between pro-(IL-8) and anti-inflammatory (IL-10) cytokine variants and their serum levels and *H. pylori*-related gastric carcinogenesis in northern India. *Meta Gene*, 6: 9-16.
9. Dinarello CA (1996). Biologic basis for interleukin-1 in disease. *Blood*, 87 (6): 2095-147.
10. Ramis IB, Vianna JS, Halicki PCB, et al (2015). Relationship of interleukin-1B gene promoter region polymorphism with *Helicobacter pylori* infection and gastritis. *J Infect Dev Ctries*, 9 (10): 1108-16.
11. Lee SG, Kim B, Choi W, et al (2003). Lack of association between pro-inflammatory genotypes of the interleukin-1 (IL-1B-31 C/+ and IL-1RN* 2/* 2) and gastric

- cancer/duodenal ulcer in Korean population. *Cytokine*, 21 (4): 167-71.
12. GarciaGonzalez M, Lanas A, Santolaria S, et al (2001). The polymorphic *IL-1B* and *IL-1RN* genes in the aetiopathogenesis of peptic ulcer. *Clin Exp Immunol*, 125 (3): 368-75.
 13. Manfred Stolte M (2001). The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. *Can J Gastroenterol*, 15 (9): 591-8.
 14. Pociot F, Mølviq J, Wogensen L, et al (1992). A *TaqI* polymorphism in the human interleukin-1 β (*IL-1 β*) gene correlates with *IL-1 β* secretion in vitro. *Eur J Clin Invest*, 22 (6): 396-402.
 15. He B, Pan Y, Xu Y, et al (2012). Increased Risk for Gastric Cancer in Carriers of the Lymphotoxin- α + 252G Variant Infected by *Helicobacter pylori*. *Genet Test Mol Biomarkers*, 16 (1): 9-14.
 16. Zhang BB, Wang J, Bian DL, Chen X-Y (2012). No association between *IL-1 β -31 C/T* polymorphism and the risk of duodenal ulcer: a meta-analysis of 3,793 subjects. *Hum Immunol*, 73 (11): 1200-6.
 17. He BS, Pan YQ, Xu YF, et al (2011). Polymorphisms in interleukin-1B (*IL-1B*) and interleukin 1 receptor antagonist (*IL-1RN*) genes associate with gastric cancer risk in the Chinese population. *Dig Dis Sci*, 56 (7): 2017-23.
 18. Resende RG, Guimarães Abreu MHN, de Souza LN, et al (2013). Association between *IL1B* (+ 3954) polymorphisms and *IL-1 β* levels in blood and saliva, together with acute graft-versus-host disease. *J Interferon Cytokine Res*, 33 (7): 392-7.
 19. ElOmar EM, Carrington M, Chow WH, et al (2000). Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*, 404 (6776): 398-402.
 20. Hnatyszyn A, Wielgus K, Kaczmarek Rys M, et al (2013). Interleukin-1 gene polymorphisms in chronic gastritis patients infected with *Helicobacter pylori* as risk factors of gastric cancer development. *Arch Immunol Ther Exp (Warsz)*, 61 (6): 503-12.
 21. Zabaleta J, Camargo MC, Piazuelo MB, et al (2006). Association of interleukin-1 β gene polymorphisms with precancerous gastric lesions in African Americans and Caucasians. *Am J Gastroenterol*, 101 (1): 163-71.
 22. Zeng ZR, Hu PJ, Hu S et al (2003). Association of interleukin 1B gene polymorphism and gastric cancers in high and low prevalence regions in China. *Gut*, 52 (12): 1684-9.
 23. Peleteiro B, Lunet N, Carrilho C, et al (2010). Association between cytokine gene polymorphisms and gastric precancerous lesions: systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev*, 19 (3): 762-76.
 24. Xue H, Lin B, Ni P, et al (2010). Interleukin-1B and interleukin-1 RN polymorphisms and gastric carcinoma risk: A meta-analysis. *J Gastroenterol Hepatol*, 25 (10): 1604-17.
 25. Rocha GA, Guerra JB, Rocha AMC, et al (2005). *IL1RN* polymorphic gene and *cagA*-positive status independently increase the risk of noncardia gastric carcinoma. *Int J Cancer*, 115 (5): 678-83.