SHORT PAPER

Association of Interleukin-1 Gene Polymorphism with Risk of Gastric and Colorectal Cancers in an Iranian Population

Mohammad Hadi Abbasian[1], Bahare Abbasi[1, 2], Nafiseh Ansarinejad[2, 3], Ali motevalizadeh Ardekani[4], Esmaeil Samizadeh[5], Katayoun Gohari Moghaddam[5], Mahmood Reza Hashemi[6]*

[1]Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology, [2]Cancer Pharmacogenetics Research Group (CPGRG), Iran University of Medical Sciences, [3]Department of Hematology and Oncology, Hazrat-e Rasool Akram University Hospital, Iran University of Medical Sciences, [4]Department of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, [5]Department of the Pathology, [6]Department of Gastroenterology, Imam Reza hospital, Aja University of Medical Sciences, Tehran, Iran.

ABSTRACT

Background: Chronic inflammation is associated with neoplasms and several types of cancer. Therefore, polymorphisms in the inflammation-related genes could modify the cancer susceptibility. **Objective:** To investigate the associations between IL-1RN VNTR and rs419598 polymorphisms in IL-1 receptor antagonist (IL-1ra) and colorectal cancer (CRC) and gastric cancer (GC) in an Iranian population. **Methods:** In this study, 126 cancer cases (91 CRC and 35 GC) and 97 healthy controls were included. Genotyping of IL-1RN VNTR and rs419598 was performed by PCR amplification and PCR-RFLP, respectively. Logistic regression was applied to identify the independent risk factors for colorectal and gastric cancers by computing the odds ratio (OR) and 95% confidence intervals (95% CI). All statistical analyses were performed using the SPSS statistical software. **Results:** There were significant differences between cancer groups and control group concerning the frequency of A1/A2 genotypes in IL-1RN VNTR polymorphism. The carrier status of IL-1RN* 2 allele was associated with increased risk of CRC (p = 0.0003; OR = 0.02; 95% CI: 0.491-0.85) and GC (p = 0.0006; OR = 0.106; 95% CI: 0.321-0.035). Also, the homozygous ILRN *2/*2 genotype was associated with increased risk of gastric cancer (p = 0.04; OR = 0.133; 95% CI: 0.020-0.908). There was no association between different alleles of rs419598 and CRC and GC. **Conclusion:** This study demonstrates an association between the carrier status of IL-1RN* 2 and CRC and GC in an Iranian population.

Abbasian M, et al. Iran J Immunol. 2018; 15(4):321-328.

Keywords: Colorectal Cancer, Genetic Polymorphisms, Inflammations, Interleukin 1 receptor antagonist

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*Corresponding author: Dr. Mahmood Reza Hashemi, MD, Assistant professor, Department of Gastroenterology, Aja University of Medical Sciences, imam Reza hospital, west fatemi street, Tehran, Iran, e-mail: hashemim891@yahoo.com

INTRODUCTION

Colorectal Cancer (CRC) as a complex disease is a major cause of cancer-related mortality in the world. In Iran, Colorectal cancer is the fourth most common cancer among men and the second among women (1). Several risk factors have been linked to CRC, including inherited susceptibility, dietary habits, lack of physical activities, and inflammatory syndromes like inflammatory bowel diseases (IBD), ulcerative colitis (UC), and Crohn's disease (CD).

Gastric Cancer (GC) remains the second cancer-related mortality worldwide. Alcohol consumption, tobacco smoking, male gender, and Helicobacter pylori are some risk factors for developing gastric cancer. Both CRC and GC carcinogenesis have a multistep process and result from a combination of genetic and environmental factors.

Chronic inflammation has been recognized as a contributing factor in the pathogenesis of colorectal, gastric, breast, and ovarian cancers. (2). Persistent inflammation is a contributing factor to the development of colorectal and gastric cancer. For instance, papillomavirus infection is associated with risk of cervical cancer (3),infection with hepatitis B increases the incidence of hepatocellular carcinoma (HCC) (4), and risk of nasopharyngeal carcinoma is increased by Epstein-Barr virus infection(5).

Helicobacter pylori (H. pylori) has infected about50% of the world's population (6, 7). H. pyloriinfection results in gastritis, peptic ulcer, and a strongriskfactor for GC. Progression of infected tissues to tumor occurs only in some H. pylori-infected individuals and appears to depend on H. pylori strains, diet, and host genetics.

Pro- and anti-inflammatory cytokines are regarded as pivotal players in tumor immunology, particularly for colorectal and gastric cancers (8, 9). Interleukin (IL)-1 is among the most important endogenous cytokines in the cytokine families (10). IL-1 has been found to contribute tochronic intestinal inflammation and tumorigenesis and tumor progression in the GI tract (11, 12). *IL-1* Gene is located at the chromosome 2q14 that contains three related genes including *IL-1A*, *IL-1B*, and *IL-1RN*, which encode IL-1a, IL-1b, and IL-1 receptor antagonist (IL-1ra), respectively.

IL-1ra, an anti-inflammatory cytokine, competitively binds to IL-1 receptors and controls the inflammatory action of IL-1 (13).

The *IL-1RN* gene has an 86-bp variable number of tandem repeats (VNTR) in the second intron (14). Five different alleles (*1 to *5) have been observed corresponding to 4, 2, 5, 3, and 6 copies of the 86-bp sequence, respectively. In another category, IL-1RN has two genotypes, long genotype (L: including alleles 1, 3, 4, and 5) and short genotype (2: allele 2 only). Many studies have shown that IL-1RN VNTR has increased the risk of gastric (15), colorectal (16), bladder (17), and cervical cancers (18).

Therefore, according to ethnic variability with regard to IL-1RN polymorphisms frequency, the aim of our study is to analyze the associations of two polymorphisms in IL-1RN VTNR and single nucleotide polymorphism in exon 2 (+2018T>C, rs419598) with the development of gastric and colorectal cancers in an Iranian population.

MATERIALS AND METHODS

Study population. The case-control study population consisted of histopathologically confirmed 126 cancer cases (91 colorectal cancer and 35 gastric cancer) and 97 healthy controls. Written informed consent was obtained from the participants. Blood and

archival formalin-fixed paraffin-embedded (FFPE) recruited from two hospitals located in Tehran, Iran (Hazrat-e Rasool and Imam Reza hospital), between February 2014 and June 2017. The ethics committee of AJA University of medical science approved the project in accordance with the tenets of the Helsinki Declaration and the national ethical guideline for medical research.

DNA extraction and genotyping. DNA samples (50 patients) were extracted from archival formalin-fixed paraffin-embedded (FFPE) by CinnaPure DNA-FFPE tissue isolation kit (sinacolneTM, Iran). Other DNA samples were extracted from 200-µl whole blood by CinnaPure DNA isolation kit (sinacolneTM, Iran).

Specific primers were designed for IL-1RN VNTR and rs419598 (Table 1). Genotyping of IL-1RN VNTR and rs419598 were performed by PCR amplification and PCR-RFLP, respectively. The characteristics of studied polymorphisms and sequences of PCR primers are shown in Table 1. PCR reactions were performed in a volume of 15 µl, containing 2 µl of the genomic DNA, 0.5µl of each specific primer in the presence of 6 µlTaq DNA Polymerase Master Mix RED (amplicon, Denmark), and 6 µl dH2O. PCR product of rs419598 was digested by MspI (HpaII,Thermo Fisher Scientific, the USA).The PCR products were then electrophoresed on a 2% agarose gel stained by ethidium bromide.Random samples were selected, 10% of experiments were repeated, and the concordance rate was 100%.

Table 1. Primers and conditions for analysis of the IL-1RN and rs419598 polymorphisms.

Polymorphism	primers	Restriction enzyme	Annealing temperature(*C*)	Length of PCR product
IL-1RN 86bp VNTR	F:CTCTGAAAGTGGATGAGACCTACAA R:GGTTAATAGAAGAGGAAGCAGCAA	-	61*c*	A2= 540 bp A4= 626 bp A1 = 712bp A3=798 bp A5=884 bp
rs419598	F: TTCCGTCTCTTGAAACTTCTACCT R:AAAGACCCAACAAGGATTAGGACAT	HpaII (MspI)	60*c*	TT = 741 CC=584 + 157 CT = 741+584+157

F: Forward, R: reverse, A: Allele

Statistical analysis

Logistic regression was used to identify the independent risk factors for colorectal and gastric cancers by computing the odds ratio (OR) and 95% confidence intervals (95% CI). A probability level (p) of less than 0.05 was considered statistically significant. TT and A1/A1 genotype were used as reference alleles for rs419598 and IL-1RN VNTR, respectively.All statistical analyses were performed using SPSS statistical software (version 24.0; SPSS Inc., Chicago, IL, USA).

Results and Discussion

The colorectal cancer group included 59 males and 32 females, with a mean age of 55.9 years (SD=12.65). A total of 24 males and 11 females with a mean age of 65.1 years make the gastric cancer group. The control group consisted of 61 males and 36 females with the mean age of 64.1 years (SD=9.4).
The genotypes and allele frequencies of IL-1RN VNTR and rs419598 polymorphisms in cancer patients and the control group and the respective cancer risks are presented in Figure. 1 and Table 2.

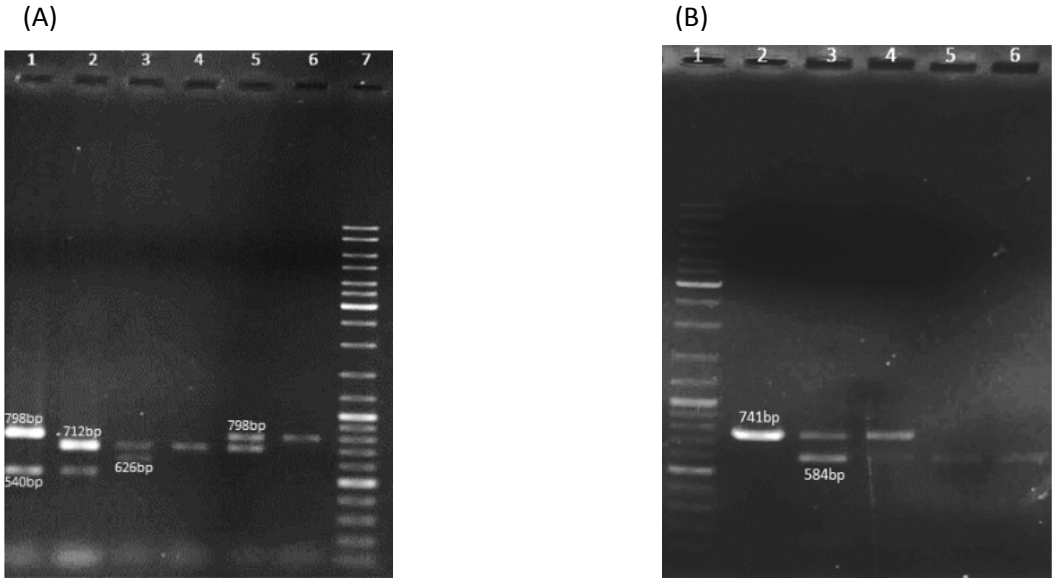

Figure 1. Genotyping of IL-1RN VNTR and rs419598 polymorphism. (A) Six different variation of IL-1RN VNTR polymorphism. Lane1, A2/A3 (798bp+540bp).lane 2, A1/A2 (712bp+540bp). Lane 3, A1/A4 (712bp+626bp). Lane 4 A1/A1 (712bp). Lane 5, A1/A3 (798bp+712bp). Lane 6, A3/A3 (798bp). Lane 7 marker(100 bp). (B) Three different variation of rs419598 polymorphism. Lane 1, marker (100bp). Lane 2, TT (741bp). Lane 3 and 4, TC (741bp, 584bp+157bp). Lane 5 and 6, CC (548bp+157bp)

There were significant differences between the two cancer groups and the control group, concerning the frequency of A1/A2 genotypes in IL-1RN VNTR polymorphism. The carriage of IL-1RN* 2 allele was associated with increased risk of colorectal ($p = 0.0003$; OR = 0.02; 95% CI: 0.491-0.85) and gastric cancers ($p = 0.0006$; OR = 0.106; 95% CI: 0.035-0.321). Also, the homozygous ILRN *2/*2 genotype was associated with increased risk of gastric cancer ($p = 0.04$; OR = 0.133; 95% CI: 0.020-0.908). There was no association between different alleles of rs419598 and colorectal and gastric cancers (Table 2).

Table 2. The distributions of IL-1RN 86bp VNTR and rs419598 genotypes and the respective colorectal and gastric cancer risks.

Polymorphism	*Allele and genotype*	*Gastric cancer patients N (%)*	*Colorectal cancer patients N (%)*	*Control*	*Gastric cancer VS control*		Colorectal cancer VS control	
					OR (95 % CI)	P-value*	OR (95 % CI)	P-value*
IL-1RN 86bp VNTR	A1/ A1	16(45.7)	48 (52.7)	75(77.3)	-	1.0	-	1.0
	A1/ A2	15(42.9)	25(27.5)	8(8.2)	0.106(0.035-0.321)	0.006	0.02 (0.491-0.85)	0.0003
	A1/A3	1(2.9)	11(12.1)	7(7.2)		0.7	0	1
	A2/A2	3(8.6)	6(6.6)	4(4.1)	1.48(0.170-12.99)	0.04	0.4(0.148-1.123)	0.083
	A2/A3	-	1(1.1)	1(1.0)	0.133(0.020-0.908)	-	0.64(0.039-10.476)	0.7
	A1/A4	-	-	1(1.0)	-	-	-	-
	A3/A3	-	-	1(1.0)	-	-	-	-
rs419598	TT	21(60.0)	51(56.0)	66(68)	-	1.0	-	1.0
	TC	14(40.0)	33(36.3)	27(27.8)	1.29(0.460-3.447)	0.6	0.632(0.338-1.182)	0.151
	CC	0.0	7(7.7)	4(4.1)	-	0.99	0.442(0.123-1.591)	0.211

Chronic inflammation plays an important role in the initiation, progression, and spread of many types of cancer including gastric, colorectal, and ovarian cancers (19).
Interleukin-1 receptor antagonist (IL-1Ra) as a member of Interleukin-1 cytokine family regulates immune and inflammatory responses (20).
To the best of our knowledge, this is the first study that investigates the role of IL-1RN VNTR and rs419598 polymorphisms in tumorigenesis of colorectal and gastric cancers in an Iranian population.
In our study, the IL-1RN* 2 allele was associated with increased risk of gastric cancer (p=0.0004) (Table 2).Three meta-analyses have reported a positive relationship between IL-1RN VNTR polymorphism and gastric cancer. Peleteiro et al. showed that IL-1RN *2 increased the risk of gastric precancerous lesions (21). Xue et al. reported an association between the allele IL-1RN*2 and increased risk of developing gastric carcinoma among Caucasians but not among Asians or Hispanics (22). Ying Zhang et al. reported that IL-1RN*2 genotype was associated with the increased risk of gastric cancer, and with increased cancer in Asian populations (23). In contrast, Kamangaret al. demonstrated the lack of any association between IL-1RN VNTR polymorphism and gastric tumorigenesis risk in western countries (24).
Furthermore, the carriage of IL-1RN* 2 alleles was associated with an increased risk of colorectal cancer in our study. The allele IL1RN*3 of VNTR variant was reported to

significantly increase the risk of colorectal cancer (25). However, in our study, we have not observed such an association with IL1RN*3 (Table 2).
In the present study, the frequency of 2/2 homozygous was 6.6% for colorectal cancer, 5.6 % for gastric cancer, and 4.1% for the control group. Comparison of results from our study for 2/2 homozygous frequency in gastric cancer patients with other studies demonstrates a difference of 28% with the European Caucasian (26) and 15% with the Brazilian (27) and 5% with the American population (16). The 2/2 homozygous frequency in another study conducted in an Iranian population was reported to be 5.2% in control groups (28).This contradictory result may be related to genetic differences in gene polymorphism between studied populations.
IL-1RN *2 genotype amplified inflammatory response and caused gastric atrophy that leading to the intestinal type of gastric cancer. IL-1RN *2 is associated with an increased level of pro-inflammatory cytokine IL-1B. IL-1B as a host factor initiates and regulates inflammatory response to H. pylori infection. IL-1B as an important contributor to genetic risk for H. pylori-induced the gastric ulcer in an Iranian population (29).
Several studies have considered rs419598 polymorphism as a risk marker for fibrosing alveolitis (30), idiopathic pulmonary fibrosis (31), Barrett's esophagus, and esophageal cancer (32).In our study, no associations were found between TT, TC, and CC alleles and risk of colorectal and gastric cancers (Table 2). In contrast to our finding, Burada et al. reported that there was a significant difference in the frequency of CC genotype between patients with colorectal cancer and the control group ($p = 0.034$)(33).
Conclusion: Although GC incidence rate has declined in developed countries, GC remains as one of the main public health problems in developing countries. In addition, the CRC rate has increased significantly over the past three decades in Iran (34).
Study of gene polymorphisms involved in inflammatory and immune response could reveal the mechanism behind inflammation-related cancer and contribute to a cancer diagnosis in early stages. Our study has identified an association between carriage of IL-1RN* 2 and gastric and colorectal cancers in an Iranian population. However, further investigations of these two polymorphisms in larger sample size are needed to confirm our results.

ACKNOWLEDGEMENTS

This work was funded by the Aja University of Medical Sciences (Grant Number: 995786). The authors also acknowledge the contribution of Javad Zamani (bioinformatics division, National Institute for Genetic Engineering and Biotechnology) for his support in statistical analysis. We are also grateful to Haniyeh Golafrouz and Hossein Golafrouz for the English revision of this manuscript.

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