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ORIGINAL

Genetic association of interleukin-1 haplotypes with gastritis and precancerous lesions in North Indians

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Abstract
Background We evaluated the association of functional variants of *IL-1* genes with the development of gastritis and precancerous lesions, which are known to be influenced by inflammatory response against *Helicobacter pylori*.
Methods After upper gastrointestinal (GI) endoscopy, 120 patients with gastritis were tested for *H. pylori* infection using rapid urease test, modified Giemsa staining and IgG anti-CagA ELISA. All patients and 243 healthy controls were genotyped for *IL-1B* (-511 C/T) and *IL-1RN* (VNTR) genes using PCR-RFLP/PCR.
Results IL-1B (-511 C/T) genotype/allele were not associated with gastritis. *IL-1RN* 1/2 genotype carriers had susceptibility to gastritis ($p = 0.025$, OR = 1.7). Individuals with the *IL-1RN* 1/1 genotype ($p = 0.05$, OR = 0.65) and *IL-1B* -511*T-*IL-1RN* *1 haplotype were at low risk for gastritis ($p = 0.043$, OR = 0.72). High secretor haplotype combinations (C1-/T2+, C1-/T1+ and T1+/T2+) did not influence neutrophilic infiltration, glandular atrophy or intestinal metaplasia.
Conclusions We identified that individuals with the *IL-1RN* 1/2 genotype had increased risk for gastritis. *IL-1B* -511*T-*IL-1RN* *1 (T1) haplotype carriers were at decreased risk for gastritis and no significant association was observed for precancerous lesions in North Indians.

Keywords Genetic association · Proinflammatory cytokines · Polymorphism · Risk

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Introduction

Individuals infected with *Helicobacter pylori* are at an increased risk of developing diseases like peptic ulcers and gastric cancer, which progresses through chronic atrophic gastritis, intestinal metaplasia and dysplasia [1]. Among bacterial factors, studies from the developed world have suggested that CagA-bearing strains of *H. pylori* are more often associated with gastroduodenal diseases than CagA-negative strains [2–4]; however, this has not been substantiated from developing countries with a high prevalence of *H. pylori* infection [5]. Although many reports show that *H. pylori* infection plays a crucial role in the pathogenesis of gastric diseases, there is a striking difference between the number of infected individuals and the number that finally result in malignancy. Hence, progression towards disease is likely to depend on host susceptibility to inflammation, which is partly influenced by genetic variations in cytokine genes [6].

Cytokine gene polymorphisms have shown an important role in determining the different patterns of *H. pylori* gastritis [7, 8]. IL-1β is a pro-inflammatory cytokine, whereas IL-1 receptor antagonist (IL-1Ra) is an anti-inflammatory cytokine that inhibits IL-1 by competing for receptor binding [9]. In *IL-1B* -511 C/T gene polymorphism, the less common allele (-511*T) has been found to be associated with gastric cancer [8, 10]. *H. pylori*-infected patients carrying the *IL-1B* -511*T allele have

increased gastric mucosal levels of IL-1β [11]. However, the *IL-1RN* gene coding for IL-1Ra cytokine contains an 86-bp tandem repeat polymorphism (VNTR) in intron 2 and the *IL-1RN* *2 allele is associated with enhanced IL-1β production [12] and gastric cancer [6, 8, 10].

The severity of inflammation is known to be governed by inflammatory infiltrates of neutrophils and lymphocytes followed by precancerous lesions like atrophy and metaplasia which may later develop into gastric malignancies. Some studies have correlated *IL-1* polymorphisms with the development of histological abnormalities like atrophy and intestinal metaplasia [13]. Recently, histopathological characteristics of gastritis have been correlated with *IL-10* haplotypes [14].

There is a known paradox regarding a high rate of *H. pylori* infection and low incidence of gastroduodenal diseases in India as compared to other Asian countries like Japan, Korea and China where low *H. pylori* infection is associated with high incidence of gastric malignant diseases. We are exploring the role of host genetic factors in this paradox [15–18]. Earlier, we did not find an association of *IL-1* and *TNF-A* genetic variants with histopathological subgroups of *H. pylori*-related gastritis in India [19]. However, these results differed from some published reports. Therefore, in the present study, we aimed to investigate the association of *IL-1B* (-511 C/T) and *IL-1RN* (VNTR) haplotypes with gastritis using case-control analysis followed by evaluation of their association with precancerous lesions.

Materials and methods

Subjects

A total of 574 patients with non-ulcer dyspepsia who underwent upper gastrointestinal (GI) endoscopy between August 2004 and December 2006 were tested for *H. pylori* infection using the rapid urease test (RUT). Of these, 120 RUT-positive patients providing histopathological evidence of gastritis were included in the present study. The patients included had dyspeptic symptoms like epigastric pain, upper abdominal discomfort, nausea and vomiting, early satiety and heartburn. Exclusion criteria were present or past history of gastric neoplasm or gastric surgery, long-term therapy with nonsteroidal anti-inflammatory drugs, liver disease and previous treatment with antibiotics or bismuth salts.

Two hundred and forty-three age-matched, healthy blood donors who had no history of dyspepsia were included as controls. Individuals with dyspeptic symptoms like epigastric pain, upper abdominal discomfort, nausea and vomiting, early satiety and heartburn, past history of any malignant disease or abdominal surgery were excluded from the control group. Demography and clinical details were obtained through a personnel interview using a standard clinical proforma. All subjects had given their informed consent for the study and the protocol was approved by the local ethics committee.

Endoscopy and histopathology

Endoscopy was performed after an overnight fast with a standard upper GI endoscope (Olympus Optical Co Ltd., Tokyo, Japan). Gastric biopsies from antrum (3) and corpus (3) were taken from each patient for histological examination. The biopsies were oriented and fixed in 10% buffered formalin. They were processed by routine techniques and 3–4 μm thick sections were cut from each block. The sections were stained using haematoxylin and eosin and the modified Giemsa stain for the detection of *H. pylori*. Histological characteristics were assessed according to the updated Sydney system [20]. A score of 0–3 (absent, mild, moderate and marked) was assigned to each of the morphological variables: neutrophilic infiltration, glandular atrophy and intestinal metaplasia.

Sample collection

The blood sample (5 ml) was taken in EDTA as well as in plain vials. The plasma was separated by centrifugation at 3000 rpm within 10 min of blood collection for the identification of bacterial virulent factor (CagA). The genomic DNA was extracted from peripheral blood leucocytes using the salting out method [21].

Serology

All serum samples were tested for the presence of IgG antibodies against the CagA antigen of *H. pylori* using standard ELISA (Genesis diagnostics, UK). In addition to the positive and negative control, six serial dilutions of the CagA antigen were included. A standard curve was constructed based upon the optical density (OD) of the control samples. An OD value ≥ 6.25 was considered as *H. pylori* positive. Repeat ELISA were performed as and when required.

Genotyping for *IL-1B* (-511 C/T) polymorphism

Polymerase chain reaction (PCR) was conducted in a total volume of 50 μl with 25 pmol of each primer; as reported by Rad et al. [13], (F) 5'-TGGCATTGATCTGGTTCATC-3' and (R) 5'-GTTTAGGAATCTTCCCACTT-3'; genomic DNA (100–150 ng); 10 mM deoxy-nucleotide triphosphates, 10x Taq polymerase buffer (5 μl) and 1.5 units of Taq DNA

polymerase (Bangalore Genei, India). PCR conditions were as follows: initial denaturation at 95°C for 5 min, 35 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s; followed by final extension of 72°C for 5 min. PCR products were digested by restriction endonuclease *Aval* (MBI Fermentas) at 37°C overnight and then analysed by 10% polyacrylamide gel electrophoresis. Genotypes were coded as follows: 304 bp for T/T; 190 and 114 bp for C/C; 304+190 and 114 bp for C/T genotype.

Genotyping for *IL-1RN* (86 bp VNTR) polymorphism

PCR reaction was carried out in a total volume of 50 µl, containing genomic DNA (100–150 ng); 10 pmol of each primer; 10 mM deoxy-nucleotide triphosphates, 10x Taq polymerase buffer (5 µl) and 1.5 units of Taq DNA polymerase (Bangalore Genei, India). The primers for the *IL-1RN* VNTR (86 bp) in intron 2 were (F) 5'-CTC AGC AAC ACT CCT AT-3' and (I) 5'-TCC TGG TCT GCA GGT AA-3' according to Tarlow et al. [22]. PCR amplification was performed under the following conditions: denaturing step at 95°C for 5 min, 35 cycles of 95°C for 30 s, 58°C for 30 s and 72 °C for 30 s and one cycle of extension at 72°C for 10 min. The PCR products were separated by electrophoresis on 2% agarose gel and further stained with ethidium bromide. Allele 1 (4 repeats) was 410 bp, allele 2 (2 repeats) 240 bp, allele 3 (3 repeats) 325 bp, allele 4 (5 repeats) 500 bp and allele 5 (6 repeats) 595 bp. Gel documentation was done by an Alphaimager‘ 1220 (Alpha Innotech Corporation, USA).

Quality control

Quality control assessment was done at every step of the study. The amount of isolated DNA was of good quality (absorbance 260 nm/280 nm ratio > 1.75). One sample with known genotype and a reagent blank were included after every 20 samples in the PCR. A 50 base-pair marker was included during electrophoresis. Twenty percent of samples from patients and controls including each genotype were re-genotyped by other laboratory personnel; no discrepancy was found even after sequencing the randomly selected 5% samples.

Statistical analysis

The proposed sample size was calculated using the QUANTO Version 1 program (http://hydra.usc.edu/gxe). The desired power of our study was set at 80% with a significance level of 0.05 in a two-sided test. In the inheritance model, we chose the log-additive one, which is the most suitable model in polygenic diseases. By means of the QUANTO program, our sample was considered adequate to study both polymorphisms.

Data were analysed using the statistical software SPSS version 11.5. Direct gene counting method was used to determine the frequency of genotypes and alleles. The χ^2 test or Fisher's exact test was used to determine differences in frequencies. A p-value < 0.05 was considered as significant. The Hardy–Weinberg equilibrium and the haplotype frequency estimation calculated from the observed genotypes were tested using Arlequin software version 2.000 [23]. The allele and genotype frequencies of *IL-1RN* (VNTR) and haplotype estimated distributions between *IL-1B* (-511 C/T) and *IL1-RN* (VNTR) were evaluated using the Monte Carlo Style CLUMP analysis program for the presence of multivariant alleles (when the standard χ^2 test on the relevant contingency table was invalid because of small expected values) [24]. Age variable was expressed as mean ± SD.

Results

Demographic profile and *H. pylori* status

The study included 120 patients (males: 57.5%) and 243 controls (males: 65.0%). There was no significant difference in the mean age of patients (36.17 ± 12.03) and controls (38.61 ± 11.04). All subjects were screened by RUT, ELISA and modified Giemsa stain to detect *H. pylori* infection (Table 1).

Table 1 Demographic profile and *H. pylori* status in gastritis patients

Demographic profile	Patients	Controls
Number	120	243
Age[a]	36.17 ± 12.03	38.61 ± 11.04
Gender		
Male, *n* (%)	69 (57.50)	145 (65.02)
Rapid urease test		
Positive, *n* (%)	120 (100)	
Anti-CagA antibody status		
Positive (%)	67.5	63.5
Modified Giemsa stain		
Positive (%)	71.5	

[a] Mean ± SD

IL-1B (-511 C/T) gene polymorphism

In controls, frequencies of all tested genotypes and alleles were in Hardy–Weinberg equilibrium. No statistically significant difference in genotype and allele frequencies of *IL-1B* (-511 C/T) was observed between gastritis patients and controls (Table 2). Further, stratification of data according to gender did not show association with disease (data not shown).

IL-1RN (86 bp VNTR) polymorphism

The genotype and allele frequency distributions of *IL-1RN* (86 bp VNTR) polymorphism between cases and controls are shown in Table 3. Genotype 1/2 was significantly ($p = 0.025$) associated with risk for gastritis (OR = 1.7, 95% CI: 1.07–2.72). On the other hand, 1/1 genotype frequency was lesser in patients (50.84%) than controls (61.32%) and it provided the resistance for gastritis ($p = 0.051$; OR = 0.65, 95% CI: 0.42–1.00). Further, stratification of data according to gender showed that genotype 1/2 was ($p = 0.061$) associated with risk in male patients (OR = 1.77, 95% CI: 0.97–3.22) (data not shown).

Haplotype estimation

There was no linkage disequilibrium between *IL-1B* (-511 C/T) and *IL-1RN* (VNTR) polymorphisms in patient ($c^2 = 5.29$, $p = 0.15$) and control ($\chi^2 = 2.25$, $p = 0.52$) populations. Seven different haplotypes like C1, C2, C3, T1, T2, T3 and T4 were possible. Odds ratios for risk estimation were calculated only for those haplotypes that had frequency > 10%. *IL-1B* -511*T-*IL-1RN**1 (T1) haplotype was associated with resistance to gastritis (OR = 0.72, 95% CI: 0.53–1.0, $p = 0.043$) (Table 4).

Association of *IL-1B* (-511 C/T) and *IL-1RN* (VNTR) haplotypes with precancerous lesions

The influence of *IL-1* gene polymorphisms on the neutrophilic infiltration and development of precancerous lesions (glandular atrophy and intestinal metaplasia) was studied (Table 5). From seven haplotypes, different combinations were created. High secretor haplotype combinations (C1-/T2+, C1-/T1+ and T1+/T2+) did not influence neutrophilic infiltration, glandular atrophy or intestinal metaplasia.

Table 2 IL-1B (-511C/T) polymorphism: allele and genotype frequencies in gastritis patients and controls

	Patients n^a (%)	Controls n^a (%)	*p*-value
Genotype			
CC	19 (15.83)	26 (10.70)	0.163
CT	64 (53.33)	132 (54.32)	0.859
TT	37 (30.84)	85 (34.98)	0.384
Allele			
C	102 (42.50)	184 (37.86)	0.229
T	138 (57.50)	302 (62.14)	0.229

[a] Total gastritis patients 120 and controls 243 (genotypes) and total chromosome number patients 240 and controls 486 (alleles)

Table 3 IL-1RN (VNTR) polymorphism: allele and genotype frequencies in gastritis patients and controls

	Patient n^a (%)	Control n^a (%)	*p*-value	OR (95% CI)
Genotypes				
1/1	61 (50.84)	149 (61.32)	0.051	0.65 (0.42–1.00)
1/2	45 (37.50)	64 (26.34)	0.025	1.71 (1.07–2.72)
1/3	6 (5.0)	5 (2.06)	0.126	2.50 (0.75–8.35)
1/4	0 (0.0)	1 (0.41)	0.481	1.00 (0.99–1.01)
2/2	6 (5.0)	23 (9.46)	0.170	0.53 (0.21–1.34)
2/3	1 (0.83)	0 (0.0)	0.155	0.99 (0.98–1.01)
2/4	1 (0.83)	0 (0.0)	0.155	0.99 (0.98–1.01)
3/3	0 (0)	1 (0.41)	0.481	1.00 (0.99–1.01)
Alleles				
1	173 (72.09)	368 (75.53)	0.290	1.21 (0.85–1.71)
2	59 (24.57)	110 (22.62)	0.559	0.90 (0.63–1.29)
3	7 (2.92)	7 (1.44)	0.174	0.49 (0.17–1.40)
4	1 (0.42)	1 (0.41)	0.202	0.24 (0.02–2.63)

[a] Total gastritis patients 120 and controls 243 (genotypes) and total chromosome number patients 240 and controls 486 (alleles)
#CLUMP (T1): $\chi^2 = 14.197$, p = ns (for genotypes), #CLUMP (T1): $\chi^2 = 2.623$, p = ns (for alleles)
#Chi square test calculated by CLUMP statistical package, when multiple allele variants are present. T1 referred to a (2x6) contingency table

Table 4 Haplotype frequency distribution of IL-1B (-511 C/T) and IL-1RN (VNTR) in patients and controls

Haplotype	Patients n^a (%)	Controls n^a (%)	*p*-value	OR (95% CI)
IL-1B -511*C-IL-1RN *1 (C1)	79 (32.60)	140 (28.80)	0.307	1.2 (0.85–1.7)
IL-1B -511*T-IL-1RN *1 (T1)	95 (39.50)	232 (47.60)	0.043	0.7 (0.53–1.0)
IL-1B -511*C-IL-1RN *2 (C2)	19 (7.60)	32 (6.60)	0.647	1.2 (0.63–2.1)
IL-1B -511*T-IL-1RN *2 (T2)	40 (17.00)	75 (15.40)	0.568	1.1 (0.74–1.7)
IL-1B -511*C-IL-1RN *3 (C3)	5 (2.30)	3 (0.60)	0.075	–
IL-1B -511*T-IL-1RN *3 (T3)	1 (0.60)	3 (0.80)	0.989	–
IL-1B -511*T-IL-1RN *4 (T4)	1 (0.40)	1 (0.20)	0.610	–

[a] Total chromosome number patients 240 and controls 486
CLUMP (T1): $\chi^2 = 6.85$, p = ns (for haplotypes)
Odds ratio was calculated for haplotypes *C1*, *T1*, *C2* and *T2* (frequency > 10%)

Table 5 Frequencies of haplotype combinations (presence/absence) in patients with different degrees of neutrophilic (N) infiltration, glandular atrophy (G) and intestinal metaplasia (IM)

Haplotype	Neutrophilic infiltration			Glandular atrophy			Intestinal metaplasia		
	Absent (n = 39)	Present (n = 81)	*p*-value	Absent (n = 57)	Present (n = 63)	*p*-value	Absent (n = 96)	Present (n = 24)	*p*-value
C1+/T1–	6	12	0.767	6	12	0.380	16	2	0.462
C1–/T1+	13	20		16	17		25	8	
C1+/T2–	18	38	0.607	26	30	1.000	46	10	0.140
C1–/T2+	9	14		10	13		15	8	
T1–/T2–	6	13	0.761	7	12	0.399	16	3	0.732
T1+/T2+	10	27		19	18		29	8	

C1+/T2– patients who were C1 (haplotype) positive and T2 negative, *C1–/T2+* patients who were C1 negative and T2 positive, *C1+/T1–* patients who were C1 positive and T1 negative, *C1–/T1+* patients who were C1 negative and T1 positive, *T1–/T2–* patients who were T1 and T2 negative, *T1+/T2+* patients who were T1 and T2 positive

Discussion

Current evidence suggests that the IL-1 cytokine family is a complex self-regulating system; its members have been proposed as candidate genes in inflammatory disorders [25–27]. In the present study, we have evaluated the association of *IL-1B* (-511 C/T) and *IL-1RN* (VNTR) polymorphisms with alleles, genotypes and haplotypes in gastritis patients and healthy controls. We did not observe any significant association of *IL-1B* (-511 C/T) polymorphism with gastritis at allele and genotype levels. In the *IL-1RN* (VNTR) polymorphism, association of the *IL-1RN* 1/2 genotype with a 1.7-fold risk for gastritis was observed. In haplotype analysis, *IL-1B* -511*T-*IL-1RN**1 (T1) haplotype carriers had resistance (low risk) for gastritis.

In the present study, the *IL-1B* polymorphism results corroborate with those of the study by Kato et al. [28], who also failed to demonstrate a positive association between the *IL-1B* -511 polymorphism and gastric cancer in a Japanese cohort. Furuta et al. [29] reported that the *IL-1B* -511TT genotype was associated with high gastric juice pH in *H. pylori*-infected subjects, whereas no association with disease was observed. However, a study by Rad et al. [14] showed that carriers of *IL-1B* -511*T alleles were associated with more severe gastric inflammation than non-carriers. Santtila et al. [12] reported that the homozygous state (*IL-1RN* 2/2) of allele*2 is a higher producer of IL-1β than the heterozygous (*IL-1RN* 1/2) or wild-type homozygous states (*IL-1RN* 1/1). Therefore, in the present study, the presence of one *2 allele dose in the *IL-1RN* 1/2 genotype (higher producer than the *IL-1RN* 1/1 genotype) subjects had a risk for gastritis. Initially, El-Omar et al. [6] estimated the increased odds ratios for individuals having the *IL-1B* -511TT and *IL-1RN* 2/2 genotypes of developing gastric cancer (1.6 and 2.9, respectively). This association was subsequently confirmed in other populations of the western world. However, other published reports, including ours [19], showed that the relationship between the *IL-1B* gene polymorphism and gastritis does not hold true in studies from Asia. A possible explanation for the discordance of Western and Asian findings could be related to ethnicity and/or geographical variations.

Earlier studies suggested the importance of the *IL-1* haplotype reflecting differential regulation of IL-1Ra expression by IL-1β and coordinated effects of polymorphisms that regulate IL-1 bioactivity *in vivo* [30]. We found a reduced risk for gastritis in subjects carrying the T1 haplotype. The reason is that the homozygous state (*IL-1RN* 1/1) or *IL1RN**1 allele in combination with the *IL-1B* *T allele is associated with higher levels of IL-1Ra

in vitro and thus individuals with higher levels of IL-1Ra may be protected against the pro-inflammatory effects of the IL-1 cytokine [25]. Furthermore, the frequency of T1 haplotype is ~48%, higher than the frequency of the *IL-1RN* 1/2 genotype (26%) in our control population. Therefore, haplotype effect (low risk) seems to dominate over genotype effect (increased risk). Previous studies had also postulated that it is not the *IL-1RN* genotype but the haplotype of the *IL-1RN* and *IL-1B* genes that play roles in modulating the susceptibility to certain disease states [31].

H. pylori infection provokes remarkable infiltration of neutrophils with severe damage to gastric epithelium, which may lead to atrophy [32], and may later develop into a gastric ulcer and/or intestinal metaplasia, dysplasia and finally gastric cancer [1]. Progression of atrophic gastritis and intestinal metaplasia into cancer has been also demonstrated in patients after *H. pylori* eradication, suggesting that a particular genetic profile makes a person susceptible to precancerous lesions despite elimination of *H. pylori* [33]. Therefore, we looked for an association of proinflammatory *IL-1* genetic profile with precancerous lesions. We observed that different haplotype combinations of *IL-1B* -511*T-*IL-1RN* *1 (T1) and T2 did not influence neutrophilic infiltration or precancerous lesions including atrophy and intestinal metaplasia. Earlier, we also did not observe an association of *IL-1* and *TNF-A* gene polymorphisms with histopathological severity of gastritis [19]. Our results differ from those of an earlier study by Rad et al. [13] who observed a significantly high risk for granulocytic infiltration, atrophy and metaplasia in gastritis patients carrying *IL-1* variant alleles. However, proinflammatory polymorphisms were not associated with precancerous lesions [34]. Different ethnic groups have their own history of migration, genetic drift and mating status, which may affect allele frequencies and disease susceptibility. Therefore, ethnic differences in the study populations may explain some of discrepancies (association/non-association) in these studies. Gastritis is a multifactorial phenotype; many factors, including several host genetic factors, may be involved, along with polygenic interaction, in pathogenesis. Therefore, genotyping of other pro- and anti-inflammatory cytokines is needed to understand this complex interplay in India.

In conclusion, we identified that individuals with the *IL-1B* -511*T-*IL-1RN* *1 (T1) haplotype were at low risk for gastritis and no significant association was observed for precancerous lesions in North Indians. It is speculated that Indian individuals with the *IL-1* gene polymorphism had resistance (decreased risk) to gastritis and no influence on precancerous lesions. Further, studies in more cohorts with gastric cancer and other precancerous lesions are required from India to understand this paradox.

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Conflict of interest The authors declare that they have no conflict of interest related to the publication of this manuscript.

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