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Original article

Interleukin-1β gene (*IL-1B*) and interleukin 1 receptor antagonist gene (*IL-1RN*) polymorphisms and gastric cancer risk in an Omani Arab population

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Abstract

Background. **Gastric cancer (GC) is the most common malignancy in Oman. Interleukin-1β gene (*IL-1B*) and interleukin-1 receptor antagonist gene (*IL-1RN*) polymorphisms have been associated with increased GC risk. No previous studies have examined their role in an Arab population. We tested the associations between polymorphisms of *IL1B* at positions –31, –511, and +3954 and the *IL-1RN* polymorphism [variable number of tandem repeats (VNTR) and TC polymorphism at the –2018 position] and GC in Omani Arab patients.**

Methods. **Genomic DNA was extracted from peripheral blood of 245 control subjects and 118 gastric cancer patients. The DNA samples were analyzed using the TaqMan allelic discrimination test for *IL-1B* –31, –511, and +3954 polymorphisms and *IL-1RN* –2018 polymorphism. The VNTR of *IL-1RN* was genotyped using the polymerase chain reaction followed by agarose gel electrophoresis.**

Results. **There was an association between the presence of *IL-1RN**2 allele and gastric cancer [odds ratio (OR) = 2.2, 95% confidence interval (CI) = 1.0–3.3, $P = 0.04$). The GC risk further increased to OR = 3.5 (95% CI = 1.0–11.9) in *Helicobacter pylori*-positive patients. No association was found between any of the other polymorphisms studied and GC.**

Conclusion. ***IL-1RN*** **polymorphism increased the risk of GC in an Omani Arab population, consistent with previous reports. In contrast, the *IL-1B* –31 polymorphism was not associated with an increased GC risk. These findings underscore the role of cytokine gene polymorphisms in the development of GC and further support the ethnic differences in the effect of *IL-1B* polymorphism on GC carcinogenesis.**

Key words Gastric cancer · Polymorphism · *IL-1B* · *IL-1RN* · *Arab* · *Omani*

Introduction

Gastric cancer is the second most common cancer worldwide and remains a significant global health problem, with widely varying geographical distribution [1]. It is the most common cancer in the Sultanate of Oman, with an age-adjusted annual incidence of 54/100,000 among males and 22/100,000 among females in 2002 [2].

Gastric carcinogenesis is a complex process resulting from interactions between genetic and environmental factors [3]. Dietary habits, such as smoking, alcohol consumption, and low intake of fruits or vegetables, are strongly implicated [4,5]. *Helicobacter pylori* infection is an established risk factor for gastric cancer, triggering chronic inflammation of the stomach and leading to stepwise development of the malignancy [5,6]. More recently, genetic risk factors such as polymorphisms of the interleukin-1β gene (IL-1B) and interleukin-1 receptor antagonist gene (*IL-1RN*) were associated with increased gastric cancer risk. *IL-1B* is a proinflammatory cytokine and a powerful inhibitor of gastric acid secretion that plays a major role in initiating and amplifying the inflammatory response to *H. pylori* infection [7–9]. *IL-1RN* encodes the IL-1 receptor antagonist (IL-1ra), an antiinflammatory cytokine that competitively binds to IL-1 receptors but does not elicit a response, thereby modulating the potentially damaging effects of IL-1.

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The genes of the IL-1 family, *IL-1A*, *IL-1B*, and *IL-1RN*, are clustered on the long arm of human chromosome 2. Three diallelic polymorphisms in *IL-1B* have been reported at –511, –31, and +3954 basepairs (bp) from the transcription start site [10]. The *IL-1RN* gene has a penta-allelic 86-bp variable tandem repeat in the second intron, resulting in a short allele (*IL-1RN**2, with two repeats) or long alleles (*IL-1RN**L, with three to six repeats) [11]. A single T-to-C base variation occurs at position –2018 in exon 2 of *IL-1RN*, with the rarer allele — *IL-1RN* –2018 allele 2 — in complete linkage disequilibrium with allele 2 of the intronic variable number of tandem repeats (VNTR) polymorphism [12].

Initially, El-Omar et al. reported increased risk for developing gastric cancer for individuals having the *IL-1B* –511T/–31C and *IL-1RN**2/*2 genotypes [8]. This finding was subsequently confirmed in Caucasian, Hispanic, and some Asian studies [8,9,13–15]. In contrast, other Asian studies have been less conclusive on the relation between *IL-1B* polymorphism and gastric cancer. For example, *IL-1B* –31 and *IL-1RN* polymorphisms were not associated with a risk of gastric cancer in Koreans or Taiwanese [16,17]. Also, the *IL-1RN* allele 2 gene was found to be extremely uncommon in a Japanese population, with no significant association with gastric cancer [18]. Similarly, Kato et al. found no association between the *IL-1B* –511 T allele and gastric cancer [19]. All of the above suggests that there is ethnic variability with regard to the *IL-1B* and *IL-1RN* polymorphisms frequency and their association with gastric cancer.

There have been no previous studies that examined the role of *IL-1B* and *IL-1RN* polymorphisms in gastric cancer in an Omani Arab population. Therefore, in this report we investigated the polymorphisms in the *IL-1B* gene (–31T/C, –511C/T, +3954 C/T single nucleotide changes) and in the *IL-1RN* gene VNTR polymorphism as well as *IL-1RN* –2018 T/C single nucleotide change and gastric cancer susceptibility in Omani Arab patients.

Materials and methods

Study subjects

The study population consisted of a series of consecutive unrelated gastric cancer patients diagnosed in three main hospitals in the Sultanate of Oman (Sultan Qaboos University Hospital, Royal Hospital, and Sohar Hospital) from January 2002 to May 2005. The control group was composed of subjects of the same ethnic and geographical origin as the patients. They were apparently healthy community-based subjects. The Medical Research and Ethics Committee of the College of Medicine of Sultan Qaboos University approved the study design. The study subjects gave informed consent prior to participation in the study.

Genotyping method

Blood (10 ml)was collected in an EDTA tube and stored frozen until DNA extraction. DNA was extracted from whole blood using a commercial DNA blood kit (Puregene DNA purification kit; Gentra, Minneapolis, MN, USA) and stored until used for genotyping.

The *IL-1B* –31, –511, +3954 polymorphisms and *IL-1RN* –2018 polymorphism were genotyped using Flourogenic 5-nuclease assay (TaqMan allelic discrimination test). Primers and SNP-specific dual fluorogenic probes labeled with Fam(6-carboxyflourescein) and Vic as a reporter and Tamra as a quencher (Applied Biosystems, Warrington Cheshire, UK) were used to determine the various alleles, as shown in Table 1. Polymerase chain reaction (PCR) cycling conditions (ABI PRISM 7000; Applied Biosystems) were as follows: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 62°C, 61°C, and 64°C for 1 min annealing temperatures for *IL-1B* –31, and +3954, and *IL-1RN* –2018, respectively. The different alleles were discriminated according to the fluorescence intensity of Fam and Vic.

For VNTR polymorphisms, the DNA was amplified using primers (Table 1) flanking the 86-bp tandem repeat polymorphic region in intron 2 of *IL-1RN*. The PCR was performed with 1 cycle of 5 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 50°C, and 30 s at 72°C; followed by 5 min at 72°C. PCR products were electrophoresed on a 2% agarose gel and visualized under ultraviolet light after staining with ethidium bromide. The *IL-1RN* alleles were coded as previously described: allele 1, four repeats; allele 2, two repeats; allele 3, five repeats; allele 4, three repeats; and allele 5, six repeats. The *IL-1RN* alleles were further divided into two categories: long genotype (L > three repeats; alleles 1, 3, 4, 5) and short genotype (2 = two repeats; allele 2). The genotypes were further classified as L/L, L/*2, and *2/*2 [20,21].

Helicobacter pylori *status analysis*

The *H. pylori* status was determined on serum specimens of all gastric cancer patients and 89 control subjects using enzyme linked immunosorbent assay (ELISA) for *H, pylori* immunoglobulin G (IgG) antibody (Inova Diagnostics, San Diego, CA, USA). Negative status was defined as a concentration below 20 U/ml as per the supplier's instructions.

Table 1. Primers and probes used for *IL-1B* and *IL-1RN* genotyping

Polymorphism	Primers and probes
IL-1B –31	5-CCC TTT CCT TTA ACT TGA TTG TGA AAT-3 Rev(-31) 5-GAG GTT TGG TAT CTG CCA GTT TCT-3 5-T(VIC)GC TTT TGA AAG CCA TAA AAA CAG CGA(TAMRA) G-3 5-T(FAM)CT GCT TTT GAA AGC TAT AAA AAC AGC GAG
IL-1B +3954	5-GCC TGC CCT TCT GAT TTT ATA CC-3 Rev(3954) 5-CAT CGT GCA CAT AAG CCT CGT TA-3 5-C(VIC)AG AAC CTA TCT TCT TCG ACA CAT GGG (TAMRA)A-3 5-T(FAM)TC AGA ACC TAT CTT CTT TGA CAC ATG GG(TAMRA)A-3
IL-1RN –2018	5-GGG ATG TTA ACC AGA AG ACCT TCT ATCT Rev(2018) 5-CAA CCA CTC ACC TTC TAA ATT GAC ATT 5-A(FAM)AC AAC CAA CTA GTT GCT GGA TACT TG CA(TAMRA)A-3 Vic label(2018) 5-A(VIC)CA ACC AAC TAG TTG CCG GAT ACT TG(TAMRA)C-3
IL-1RN (VNTR)	5-CTCAGCAACACTCCTAT-3 Rev (IL1RA) 5-TCCTGGTCTGCAGGTAA-3

Statistical analyses

The genotype distributions of various polymorphic loci in controls were compared to that expected from the Hardy-Weinberg equilibrium by χ^2 tests. The difference in frequency distributions of genotypes between the patient and the control groups was also tested by χ^2 test. Age- and-sex adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression analysis. $P < 0.05$ was considered statistically significant. Analysis of data was performed using SPSS 10.0 software.

Results

A total of 118 gastric cancer patients and 245 healthy controls were included. The intestinal and diffuse histology subtypes were found in 63 and 55 gastric cancer specimens, respectively. The mean age of the gastric cancer patients was 56.5 years (median ± SD 56.0 ± 12.3) and 29.3 years (median ± SD 26.0 ± 10.8) for the control population. The percentage of males was 55.1% in the cancer group and 56.9% among the control subjects. Our control subjects were closely matched in terms of sex, but they were younger than the patients. To minimize the possible confounding effects caused by age, we adjusted the odd ratio calculations for age and sex as described previously [20,22].

Genotype frequencies and gastric cancer risk

The frequencies of *IL-1B* and *IL-1RN* genotypes are shown in Tables 2 and 3. *IL-1RN* and *IL-1B* allelic distributions did not deviate significantly from those expected in a Hardy-Weinberg equilibrium for control subjects. The frequency of *IL-1B* -31 C allele in the Omani population was 48% compared with 34% and 51% in Caucasian and Asian populations, respectively [8,16,22]. *IL-1B* –511 polymorphism was studied in all gastric cancer patients and 156 controls. Linkage disequilibrium between the T-to-C transition at *IL-1B* –31 and the C-to-T transition at *IL-1B* –511 was confirmed in Omani patients and their controls (r^2 for patients = 0.88; r^2 for controls = 0.93) (data not shown). *IL-1B* +3954 T allele frequency was 30% compared to 23% and 6% in Caucasian and Asian populations, respectively [20,22]. The frequency of the *IL-1RN**2 allele was 15% compared with 26% and 6% in Caucasian and Asian populations, respectively [16,22]. The single nucleotide polymorphism at T-to-C basepair change at position –2018 in exon 2 is in linkage disequilibrium with allele 2 of the intronic VNTR polymorphism in Omani gastric cancer patients ($r^2 = 0.98$) and controls ($r^2 = 1.0$), as shown in Table 3.

The carriage of *IL-1RN** 2 allele was associated with increased risk of gastric cancer (OR = 2.2, 95% CI = 1.1–2.7, $P = 0.04$) (Table 3). The homozygous *ILRN* *2/*2 genotype was found in only three patients and was not associated with gastric cancer ($P = 0.3$). The heterozygote genotype *2/L was associated with increased risk, with OR = 2.6 (95% CI = 1.2–4.6, $P = 0.015$). There was no significant association between the other studied

Table 2. *IL-1B* genotypes and gastric cancer association risk in gastric cancer patients and control subjects

Genotype	Patients (n = 118)	Control (n = 245)	Odds ratio[a] (95% CI)
IL1B (–31)			
TT	31 (26.3%)	70 (28.6%)	1
CC	27 (22.9%)	59 (24.1%)	1.4 (0.5–3.7)
CT	60 (50.8%)	116 (47.3%)	0.9 (0.4–2.0)
C/C + T/C	87 (73.7%)	175 (71.4%)	1.0 (0.5–2.2)
Allele frequency C	0.48	0.48	
IL1B(–3954)			
CC	61 (51.7%)	127 (51.8%)	1
TT	9 (7.6%)	27 (11.1%)	0.7 (0.2–2.8)
TC	48 (40.7%)	91 (37.1%)	1.3 (0.6–2.8)
TT/TC	57 (48.3%)	118 (48.2%)	1.2 (0.6–2.4)
Allele frequency T	0.28	0.30	

CI, confidence interval
[a] Age- and sex-adjusted odds ratio

Table 3. *IL-1RN* genotype studied by VNTR polymorphism and single nucleotide: 2018 polymorphism and gastric cancer association risk in gastric cancer patients and control subjects

Genotype	Patients (n = 118)	Control (n = 245)	Odds ratio[a] (95% CI)
IL-1B –2018			
TT	73 (61.9%)	181 (73.9%)	1
CC	2 (1.7%)	11 (4.5%)	0.4 (0.2–2.8)
TC	43 (36.4%)	53 (21.6%)	2.6 (1.2–5.7)
TC/CC	45 (38.1%)	64 (26.1%)	2.2 (1.14.7)
Allele frequency C	0.2	0.15	
IL-1RN			
LL	73 (61.9%)	181 (73.9%)	1
2/2	3 (2.5%)	11 (4.5%)	0.4 (0.2–3.3)
2/L	42 (35.6%)	53 (21.6%)	2.6 (1.2–4.6)
2/L + 2/2	45 (38.1%)	65 (26.1%)	2.2 (1.1–4.7)
Allele frequency 2	0.2	0.15	

[a] Age- and sex-adjusted odds ratio

polymorphisms and gastric cancer risk, as the calculation of odds ratio did not reach statistical significance with any genotype (Table 2).

Combined IL-1RN *and* IL-1B *genotypes and gastric cancer risk*

The age- and sex-adjusted odds ratios of the combined genotype analysis of *IL-1B* –31 or +3945 and *IL-1RN* were estimated as previously described [21]; the results are shown in Table 4. A significant gastric cancer risk association (OR = 7.9) was observed in the combined genotype of homozygote wild *IL-1B* –31 TT and *IL-1RN* *2/L (P = 0.02). There was a twofold increase in gastric cancer risk for *IL-1B* –31 *C allele carriers and *IL-1RN* *2 allele carriers, but it was not statistically significant (P = 0.17). There was also about a threefold increase in gastric cancer risk for the combined genotype of homozygous wild *IL-1B* –3954 CC and *IL-1RN* *2 and combined genotype *IL-1B* –3954 *T allele carrier and *2 allele carrier (P = 0.05).

Interaction between H. pylori *and* IL-1B *genotypes*

All 118 gastric cancer patients and 89 control subjects were tested for *Helicobacter* serology. The rates of positive serology were 56.8% for the cancer group and 59.5% for the control group. There was no change in the lack of association between *IL-1B* –31 and +3945 and gastric cancer. The risk for *IL-1RN** 2 allele carriers increased by 3.5 (95% CI = 1.0–11.9, P = 0.04) as shown in Table 5. The gastric cancer risk was more evident for

Table 4. Combined *IL-1RN* and *IL-1B –31* genotypes and gastric cancer risk association in gastric cancer patients and controls

Combined genotype		Patients (n = 118)	Control (n = 245)	Odds ratio[a] (95% CI)
IL1RN	*IL1B –31*			
LL	TT	23 (19.5%)	76 (31.0%)	1
LL	TC + CC	49 (41.5%)	105 (42.9%)	1.0 (0.4–2.6)
2/L + 2/2	TT	9 (7.6%)	6 (2.4%)	7.9 (1.3–47.9)
2/L + 2/2	T/C + CC	37 (31.4%)	58 (23.7%)	2.0 (0.7–5.6)
IL1RN	*IL1B –3954*			
LL	CC	32 (27.1%)	89 (36.2%)	1
LL	CT + TT	44 (37.3%)	92 (37.6%)	2.0 (0.9–4.9)
2/L + 2/2	CC	25 (21.2%)	43 (17.6%)	2.8 (1.0–7.9)
2/L + 2/2	TC + TT	17 (14.4%)	21 (8.6%)	3.3 (1.0–10.6)

[a] Age- and sex-adjusted odds ratio

Table 5. Gastric cancer risk association with *IL-1RN* polymorphism in 118 gastric cancer patients and 89 control subjects according to *Helicobacter pylori* status

Parameter	*H. pylori* positive		Odds ratio[a] (95% CI)	*H. pylori* negative		Odds ratio[a] (95% CI)
	Cases	Controls		Cases	Controls	
L/L	42	46	1	31	22	1
2* Carrier	25	7	3.5 (1.0–11.9)	21	13	1.4 (0.5–4.3)
P	0.04					0.5

[a] Age- and sex-adjusted odds ratios

IL-1RN 2/L (OR = 5.4, 95% CI = 1.4–20.8, P = 0.01) (data not shown).

Interaction between intestinal histology subtype and IL-1B *genotypes*

The subclassification of gastric cancer histology into intestinal and nonintestinal did not show any effect on gastric cancer risk for any of the genotypes studied (data not shown).

Discussion

We investigated the association between GC and *IL-1B* –31, –511, +3954 polymorphisms and single base –2018 polymorphisms and the VNTR polymorphism of *IL-1RN* gene in an Omani Arab population, an ethnic group in which the association between gastric cancer and these polymorphisms has not been studied previously.

Our results showed that the presence of *IL-1RN**2 allele independently increases the risk of gastric cancer, with an OR of 2.2 in the Omani population. This finding is in agreement with other reports that also demonstrated that the presence of *IL-1RN**2 increased the risk of gastric cancer [8,13,14]. Most of these studies reported this association with an *IL-1RN**2 homozygous genotype; however, we found this association with a heterozygous genotype, which is consistent with four other studies from Brazil, Taiwan, and Italy that found the same association between *IL-1RN**2 allele carriers and gastric cancer [21–24]. The variation in *IL-1RN* gastric cancer predisposition risk between Asian studies and others including ours may be due in part to the variation in frequency of the *IL1-RN**2 genotype in the populations in question. The *IL-1RN**2 allele is uncommon in Asians, for whom frequencies of around 6% are reported, compared to 26% in Caucasians and 15% in our study [8,16].

The biological function of secreted *IL-1RN* is to inhibit competitively the binding of circulating *IL-1B* to cell-surface receptors and to counterbalance the potentially injurious proinflammatory effects of IL-1B. The presence of *IL-1RN**2 was associated with severe acute and chronic inflammation in the gastric mucosa [25]. Moreover, an altered *IL-1RN/IL-1B* ratio was found to be associated with a heightened and prolonged proinflammatory immune response [26,27]. This view is further supported by our data showing that the combined analysis the gastric cancer risk was particularly increased among carriers of the *IL-1RN**2 allele, who

were also homozygous for the wild *IL-1B* –31 T allele (OR = 7.9), suggesting an interaction between these two genotypes.

We found no evidence of increased gastric cancer risk among *IL-1B* –31C carriers or *IL-1B* +3954 T carriers. In other studies, *IL-1B* –31 C polymorphisms have been shown to be associated with increased risk of gastric cancer in Caucasian and Hispanic populations [8,15]. However, these results could not be reproduced in studies performed in Japanese, Korean, or Taiwanese subjects [16,17,19]. This indicates that our results are consistent with those reported in Asians but not those in Caucasians.

In our study population, the *IL-1B* –31 and *IL-1B* –511 polymorphisms suggested that these polymorphisms were tightly linked, in accord with earlier studies [8,13]. Linkage disequilibrium between the T-to-C transition at *IL-1B* –31 and the C-to-T transition at *IL-1B* –511 was confirmed in Omani patients and controls. The single nucleotide polymorphism at T-to-C basepair change at position –2018 in exon 2 is completely associated with allele 2 of the intronic VNTR polymorphism in Omani gastric cancer patients [12].

With regard to the interaction between *H. pylori* infection and *IL-1B* genotype polymorphism, we found no difference in the prevalence of *H. pylori* infection in the control and case groups, with positive serology rates of 59.5% and 56.8%, respectively. The gastric cancer risk was more evident for the heterozygous *IL-1RN* 2/L (OR = 5.4; 95% CI = 1.4–20.8). Similarly, the risk for *IL-1RN*2* carriers increased by 3.5 (95% CI = 1.0–11.9; P = 0.04). This suggests an interaction between concomitant *H. pylori* infection and *IL-1RN*2** carrier status. The immune regulatory role of the antiinflammatory cytokine of *IL-1RN* with concomitant *H. pylori* infection is implicated [28]. *H. pylori* infection in individuals carrying the *IL-1RN**2 allele results in increased production of gastric *IL-1B*, leading to severe and sustained inflammation, gastric atrophy, and hypochlorhydria — and ultimately to the development of gastric carcinoma [8,9,26].

Conclusion

The *IL-1RN* polymorphism contributes significantly to the risk of gastric cancer in an Omani Arab population, confirming the findings in Caucasians, Hispanics, and Asians. This risk is further increased with concomitant *H. pylori* infection, which underscores the role played by host genetics in *H. pylori*-related gastric carcinogenesis. However, the widely reported association between *IL-1B* –31/–511 polymorphism and gastric cancer was not established in the Omani Arab population, supporting the ethnic differences in the effect of *IL-1B* polymorphism on gastric cancer carcinogenesis.

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