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Role of bacterial and genetic factors in gastric cancer in Costa Rica

Sergio A Con, Hiroaki Takeuchi, Gil R Con-Chin, Vicky G Con-Chin, Nobufumi Yasuda, Reinaldo Con-Wong

Sergio A Con, Gil R Con-Chin, Vicky G Con-Chin, Reinaldo Con-Wong, Centro Digestivo Doctores Con-Mediplaza, Pavas 245-1200, San José, Costa Rica
Sergio A Con, Hiroaki Takeuchi, Department of Clinical Laboratory Medicine, Kochi Medical School, Kochi University, Nankoku-city, Kochi 783-8505, Japan
Nobufumi Yasuda, Department of Public Health, Kochi Medical School, Kochi University, Nankoku-city, Kochi 783-8505, Japan
Author contributions: Con SA designed and performed the research and wrote the paper; Takeuchi H, Con-Chin GR, Con-Chin VG and Con-Wong R contributed reagents/analytic tools; Yasuda N analyzed the data; Takeuchi H and Con-Wong R revised the paper.
Correspondence to: Dr. Sergio A Con, Centro Digestivo Doctores Con-Mediplaza, Pavas 245-1200, San José, Costa Rica. scon@gastrocolon.com
Telephone: +506-2201-7028 Fax: +506-2201-7033
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Abstract

AIM: To evaluate several risk factors for gastric cancer (GC) in Costa Rican regions with contrasting GC incidence rate (GCIR).

METHODS: According to GCIR, 191 *Helicobacter pylori* (*H pylori*)-positive patients were classified into groups A (high GCIR, *n* = 101) and B (low GCIR, *n* = 90). Human DNA obtained from biopsy specimens was used in the determination of polymorphisms of the genes coding for interleukin (IL)-1β and IL-10 by PCR-RFLP, and IL-1RN by PCR. *H pylori* DNA extractions obtained from clinical isolates of 83 patients were used for PCR-based genotyping of *H pylori cagA*, *vacA* and *babA2*. Human DNA from gastric biopsies of 52 GC patients was utilized for comparative purposes.

RESULTS: Cytokine polymorphisms showed no association with GCIR variability. However, gastric atrophy, intestinal metaplasia and strains with different *vacA* genotypes in the same stomach (mixed strain infection) were more frequently found in group A than in group B, and *cagA* and *vacA s1b* were significantly associated with high GCIR (*P* = 0.026 and 0.041, respectively). IL-1β+3954_T/C (OR 2.1, 1.0-4.3), IL-1RN*2/L (OR 3.5, 1.7-7.3) and IL-10-592_C/A (OR 3.2, 1.5-6.8) were individually associated with GC, and a combination of these cytokine polymorphisms with *H pylori vacA s1b* and *m1* further increased the risk (OR 7.2, 1.4-36.4).

CONCLUSION: Although a proinflammatory cytokine genetic profile showed an increased risk for developing GC, the characteristics of *H pylori* infection, in particular the status of *cagA* and *vacA* genotype distribution seemed to play a major role in GCIR variability in Costa Rica.

Key words: Costa Rica; Gastric cancer; *Helicobacter pylori*; Host genetic factors

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INTRODUCTION

Costa Rica has one of the highest age-adjusted incidence and mortality rates for gastric cancer[1]. In fact, this country reported the highest age-adjusted gastric cancer mortality rate in males and females over the period 1983-1997, out of a total of 30 countries, including Japan and Chile[2].

Costa Rica has regions with contrasting gastric cancer incidence rates (GCIR). Topographically, the central part of the country is predominantly composed of regions with high GCIR while coastal areas are largely characterized by low GCIR[3]. Population density varies according to geographic area. While in coastal regions the population density is around 30 persons per square km, in the central regions of San Jose and Cartago, it ranges from 140 to 270 persons per square km. Cultural, behavioral and dietary patterns are very similar throughout the country, regardless of population density[3]. The pre-

dominant ethnic group is the criollo, which has Spanish ancestry. In spite of these homogeneous patterns, the GCIR in Costa Rica shows a distinctive regional variation[4]. Several environmental factors such as the components of drinking water, soil and nutrients have been compared in contrasting GCIR regions; however, none of these factors was significantly associated with GCIR variation in the country[4,5].

The cause of gastric cancer is thought to be multifactorial. A higher incidence of gastric cancer in blood type A subjects than in those with other blood types was reported as early as the 1950's[6,7]. Several decades later, after the discovery of *Helicobacter pylori* (*H pylori*), which is a Gram-negative microaerobic bacterium that persistently colonizes the human gastric mucosa, it was reported that *H pylori*-positive subjects are believed to have a two- to three-fold increased risk of developing gastric cancer when compared with *H pylori*-negative subjects[8-11]. The risk is even higher in subjects infected with strains co-expressing the *H pylori cagA*, *vacA s1* and *babA2* genes[12-15]. Recently, cytokine gene polymorphisms of the host, IL-1β, IL-1RN and IL-10, in response to *H pylori* infection, have been associated with an increased risk for developing gastric cancer[16-21]. Moreover, it has been suggested that an interaction between a host's immunological defenses, environmental and *H pylori* virulence factors play a main role in the development of gastric cancer[22,23].

We previously reported that the presence of serum CagA antibody was found to be significantly higher in high GCIR regions than in low GCIR regions in Costa Rica, despite the fact that no significant difference was found in the prevalence of *H pylori* infection between the regions, suggesting that the *H pylori cagA* gene was associated with the development of severe gastric injury, glandular atrophy and cancer, which probably influenced the GCIR variability in the country[24]. However, further investigation is needed to demonstrate a significant association of *H pylori* and/or host factors with GCIR variability in Costa Rica.

The aim of this study was to evaluate whether host genetic factors such as interleukin (IL)-1β (-511 and +3954), IL-10 (-1082 and -592) and IL-1RN intron 2 variable number of tandem repeat (VNTR) polymorphisms in response to *H pylori* infection, and/or *H pylori cagA*, *vacA* and *babA2* genotype distribution could be associated with the GCIR variability present in Costa Rica.

MATERIALS AND METHODS

Study population

The patients in this study attended a digestive center in San Jose, Costa Rica. Out of 402 continuous dyspeptic patients who underwent upper endoscopy from January to July 2005 and from January to July 2006, a total of 191 *H pylori*-positive patients (80 males, 111 females; age range 23-76 years) were enrolled for the determination of cytokine gene polymorphisms in *IL-1β*, *IL-1RN* and *IL-10*. Clinical isolates successfully obtained from both antrum and corpus specimens of 83 patients were eventually utilized for the PCR-based genotyping of the *H pylori cagA*, *vacA* and *babA2* genes. Informed consent was obtained from each patient and the study was approved by the Ethics Committee of the institution.

In addition, gastric tissue specimens obtained from 52 consecutive *H pylori*-positive gastric cancer patients (GC group) who underwent surgical treatment at a hospital in Cartago, Costa Rica between February 2006 and March 2007, were utilized in this study to determine cytokine gene polymorphisms of the host, and were used for comparative purposes.

Based on a previous study[4], dyspeptic patients were classified into either high or low GCIR groups. Group A (high GCIR) was composed of patients belonging to regions with a GCIR in the range of 24.7-48.5/100 000 persons, while in group B (low GCIR) the incidence rates ranged from 9.8-19.9/100 000 persons. Patients belonging to regions with a GCIR of 20.0-24.6/100 000 persons were removed from the study to further distinguish group A from group B. Information on age, gender, place of origin, symptoms and medication was collected. Patients with a recent intake of proton pump inhibitors, antibiotics, non-steroidal anti-inflammatory drugs, or any drug that could alter the state of the gastric mucosa were excluded from this study. Likewise, patients with *H pylori* eradication or previous attempted eradication therapy, previous gastric surgery as well as patients with Asian ancestry were also excluded from the study.

Endoscopic and histological evaluations

Endoscopy was performed with Olympus Evis Excera 160 and 180 videoendoscopes (Olympus America Inc., San Jose, CA, USA). From each patient, five biopsies (two from the antrum, two from the corpus and one from the cisura angularis) were collected for histological examination. Two more biopsies (one from the antrum and one from the corpus) were also taken to obtain the isolates following bacterial culture.

The five biopsy samples from each of the 191 patients were conventionally fixed in 100 mL/L aqueous formaldehyde, and embedded in paraffin. Serial 3- to 4-μm sections were stained with hematoxylin and eosin for histological observation. Each biopsy specimen was evaluated independently by two experienced pathologists blinded to the endoscopic and laboratory examinations. All discrepant diagnoses were re-examined by both pathologists together in order to reach a final consensus diagnosis. All five biopsies were examined for the presence of glandular atrophy and intestinal metaplasia and were scored into one of four grades (0: none, 1: mild, 2: moderate and 3: marked) for both the antrum and the body of the stomach, according to the updated Sydney System of classification and grading of gastritis[25]. Gastric glandular atrophy was defined as the loss of gastric glands, and its replacement with fibrosis or metaplastic epithelium. Intestinal metaplasia was defined as the presence of foci where at least three neighboring gastric pits containing two or more goblet cells (in each pit) were visualized in any part of the stomach.

Table 1 PCR primers for amplification of *cagA*, *vacA* and *babA2* genes

Region	Primer	Nucleotide sequence	Reference
cagA	D008	5'-ATAATGCTAAATTAGACAACTTGAGCGA-3'	[28]
	R008	5'-TTAGAATAATCAACAAACATCACGCCAT-3'	
	cagAFnz3	5'-AAAAGCGACCTTGAAAATTCC-3'	[29]
	cagA-seqR1	5'-TAGCATAATTGTCCAATTTCGC-3'	
vacA s1	VA1-F	5'-ATGGAAATACAACAAACACAC-3'	[30]
	VA1-R	5'-CTGCTTGAATGCGCCAAAC-3'	
vacA s1a	S1A-F[1]	5'-TCTYGCTTTAGTAGGAGC-3'	[30]
vacA s1b	SS3-F[1]	5'-AGCGCCATACCGCAAGAG-3'	[30]
vacA s1c	S1C-F[1]	5'-CTYGCTTTAGTRGGGYTA-3'	[13]
vacA s2	VA1-F[1]	5'-ATGGAAATACAACAAACACAC-3'	[30]
vacA m1	VA3-F	5'-GGTCAAAATGCGGTCATGG-3'	[30]
	VA3-R	5'-CCATTGGTACCTGTAGAAAC-3'	
vacA m2	VA4-F	5'-GGAGCCCCAGGAAACATTG-3'	[30]
	VA4-R	5'-CATAACTAGCGCCTTGCAC-3'	
babA2	babA-F	5'-AATCCAAAAAGGAGAAAAAGTATGAAA-3'	[31]
	babA-R	5'-TGTTAG TGATTTCGGTGTAGGACA-3'	
	babA2-Fnc1	5'-GAAAAAACATGAAAAAACACATCCTTTCAT-3'	
This study			
	babA2-Rmn2	5'-TCTGGGTTAATGGCTTGCC-3'	

[1]Used with primer VA1-F.

Determination of H pylori infection

H pylori infection was determined by either serum antibodies to *H pylori*, rapid urease test (RUT) or histological examinations of biopsy specimens obtained from the antrum, cisura angularis and body of the stomach. Patients were considered to be infected with the bacterium if either serum antibodies to *H pylori* were found, the biopsy specimen was positive for RUT or the bacterium was observed in any of the hematoxylin and eosin-stained sections.

Extraction of human DNA and genotyping of cytokine polymorphisms

Human DNA was extracted from biopsy specimens using a DNA extraction kit (QIAamp DNA mini kit; Qiagen K.K., Tokyo, Japan), according to the manufacturer's instructions. Cytokine gene polymorphisms in *IL-1β* (-511 and +3954) and *IL-10* (-1082 and -592) were examined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis, as described previously[26,27] and visualized by 50 mL/L ethidium bromide staining on 30 mL/L agarose gels. The IL-1RN variable number of tandem repeat (VNTR) polymorphism was detected by PCR and visualized on 20 mL/L agarose gels with alleles being classified conventionally according to El-Omar *et al*[18] as follows: allele 1, four repeats; allele 2, two repeats; allele 3, five repeats; allele 4, three repeats and allele 5, six repeats. Because alleles 3, 4 and 5 were very rare, the alleles were classified into short (allele 2: *2) and long (alleles 1, 3, 4 and 5: L) alleles for statistical analysis, as described previously[14].

Isolation of H pylori from biopsy specimens and DNA extraction

The homogenized biopsy specimens were placed on *H pylori* selective agar plates (Helico VI agar, E-MS70, Eiken Chemical Co., Ltd., Japan) and cultured at 37°C under microaerobic conditions (100 mL/L CO_2) for five to seven days. The presence of *H pylori* colonies was confirmed by typical morphology, Gram staining and a positive urease test. From 83 patients, a total of 166 clinical isolates obtained from both antrum and corpus specimens were subjected to genomic DNA (gDNA) extraction using a DNA kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions.

Detection of H pylori cagA, vacA and babA2 genes by PCR

The genomic DNAs were subjected to PCR for *H pylori* genotyping analysis. Genotyping of the *cagA* gene was examined using primer pairs D008 and R008, and cagA-Fnz3 and cagA-seqR1[28,29] (Table 1). The analysis of the *vacA s* and *m* regions was carried out as previously described[13,30]. Genotyping of the *babA2* gene was examined using reported primers[31] and additional primers babA2-Fnc1 (5'-GAAAAAACATGAAAAAACACATCCTTT-CAT-3') and babA2-Rmn2 (5'-TCTGGGTTAATGGCT-TGCC-3') designed according to the following conditions: pre-heat for 2 min at 96℃, followed by 40 cycles at 96℃ for 30 s, 49℃ for 30 s, and 72℃ for 1 min. All discrepant results of *cagA* and *babA2* genotyping were confirmed by sequence analysis (Genetic Analyzer 3130 Applied Biosystems, Foster City, CA, USA) following PCR using a Big Dye Terminator v1.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Statistical analysis was performed using the Chi-square test and the Fisher's exact probability test (STATA SE (version 8) statistical software). A P-value of < 0.05 was regarded as statistically significant. Multivariate

Table 2 Characteristics of *H pylori*-positive Costa Rican patients

	Group A	Group B	*P*-value
Number of patients	101	90	
Gender (male/female)	49/52	33/57	0.81
Mean age (yr, ± SD)	50.4 ± 11.5	50.9 ± 13.6	0.99
AG-positive (%)	36 (35.6)	24 (26.7)	0.12
IM-positive (%)	17 (16.3)	6 (6.7)	0.02

analysis was performed by logistic regression (SPSS 13.0 Japanese version (SPSS Japan Inc., 2005) adjusting for gender and age. Odds ratios (OR) with 95% confidence intervals (CI) were used to study the influence of host and bacterial factors on the development of gastric cancer.

RESULTS

Comparison of gender and age of patients between groups A and B

Gender and age distribution in group A (101, 49 men, 52 women; mean ± SD, 50.42 ± 11.5 years) was not significantly different when compared with that in group B (90, 33 men, 57 women; mean ± SD, 50.87 ± 13.6 years) ($P = 0.81$ and $P = 0.99$, respectively; Table 2).

Gastric atrophy and intestinal metaplasia in groups A and B

The prevalence of gastric atrophy was higher in group A (35.6%, 36/101) than in group B (26.7%, 24/90), although the difference did not reach statistical significance ($P = 0.12$, Table 2). However, the prevalence of intestinal metaplasia was found to be significantly higher in group A (16.3%, 17/101) than in group B (6.7%, 6/90; $P = 0.02$).

Interleukin-1 and -10 polymorphisms in groups A and B

The analysis of cytokine gene polymorphisms including IL-1β-511 and +3954, IL-1RN intron 2, and IL-10-1082 and -592 did not reveal any significant difference between groups A and B (Table 3). However, when the role of cytokine polymorphisms on gastric cancer was evaluated, IL-1β+3954_T/C, IL-1RN*2/L, IL-10-592_A/A and IL-10-592_C/A were found to be individually associated with this cancer, irrespective of GCIR grouping (Table 3).

Mixed strain infection of H pylori colonized in the stomach in clinical isolates obtained from the antrum and corpus

Mixed strain infection of *H pylori* has been defined as the colonization of the same patient by *H pylori* strains harboring more than one *vacA* genotype in the same stomach[32]. The analysis of the *H pylori vacA* gene in terms of its presence/absence and genotype in each clinical isolate between the antrum and corpus in 83 patients, showed a mixed strain infection in only one patient belonging to group B and in six patients belonging to group A, of which five were diagnosed with either gastric atrophy or both gastric atrophy and intestinal metaplasia (Table 4). The *cagA* and *babA2* genes were also examined according to the same terms in those 83 patients. The prevalence of *cagA* did not differ in any of the patients while the prevalence of *babA2* differed in two patients without discordant *vacA* alleles, both belonging to group A.

H pylori cagA, vacA and babA2 genes in clinical isolates from a non-mixed infection

In the 76 clinical isolates obtained from a non-mixed infection, the prevalence of *cagA* and the prevalence of *vacA s1b* in group A (both 87.8%) were found to be significantly higher than those in group B (65.7% and 68.6%, respectively) (Table 5). A tendency for an association between *vacA m1* and GCIR variability was reported, while no significant difference was found in the prevalence of *babA2* between the groups.

Combination of cytokine polymorphisms and H pylori virulence factors in gastric cancer and non-gastric cancer patients

To investigate the influence of combined factors on the development of GC, we used the cytokine polymorphisms that were associated with GC in this study. The presence of a combination of IL-1β+3954_T/C, IL-1RN*2/L and IL-10-592_C/A slightly increased the risk of GC (adjusted OR 4.7, 1.7-13.0) when compared with patients carrying only one of the cytokine polymorphisms previously cited (Table 6). However, a combination of these polymorphisms with the addition of *H pylori vacA s1b* and *m1* genotypes, which were chosen due to their association with GC reported in a previous Costa Rican study[29], considerably increased the risk of GC (adjusted OR 7.2, 1.4-36.4). The risk was further increased when a combination of only IL-1 polymorphisms (IL-1β+3954_T/C, and IL-1RN*2/L) and *H pylori vacA s1b/m1* was evaluated (adjusted OR 9.8, 2.9-32.9).

DISCUSSION

The gastric cancer incidence rate in Costa Rica shows regional variation. Using *H pylori*-positive patients selected from high and low GCIR regions, the main objective of this study was to evaluate the potential impact of *H pylori* and/or host genetic factors on GCIR variability in Costa Rica.

The analysis of human genetic polymorphisms within the cytokine genes *IL-1β*, *IL-1RN* and *IL-10* (Table 3) as well as the ABO blood group status (data not shown) did not show any significant differences between groups A and B (high and low GCIR groups, respectively) indicating that the genetic profile of the host, including these evaluated factors, did not seem to be linked to GCIR variability in Costa Rica. It has been reported that the presence of proinflammatory cytokines induces a hypochlorhydric and atrophic response to

Table 3 Statistical analysis for several cytokine gene polymorphisms according to high gastric cancer incidence rate and gastric cancer in *H pylori*-positive Costa Rican patients

	High GCIR			Gastric cancer		
	Pos/Neg	OR (95% CI)	*P*-value	Pos/Neg	OR (95% CI)	*P*-value
Interleukin-1β-511						
T/T	28/19	1.9 (0.8-4.3)	0.136	18/47	1.6 (0.7-4.1)	0.283
T/C	50/43	1.4 (0.7-2.8)	0.317	24/93	1.2 (0.5-2.9)	0.629
C/C	23/28	1.0 reference		10/51	1.0 reference	
Interleukin-1β+3954						
T/T	2/0	-	-	-	0/2	-
T/C	56/45	1.4 (0.8-2.6)	0.237	39/101	2.1 (1.0-4.3)	0.049
C/C	43/45	1.0 reference		13/88	1.0 reference	
Interleukin-1RN intron 2						
*2/*2	20/11	2.2 (0.9-5.2)	0.078	4/31	0.7 (0.2-2.2)	0.494
*2/L	31/28	1.2 (0.6-2.3)	0.592	33/59	3.5 (1.7-7.3)	0.001
L/L	50/51	1.0 reference		15/101	1.0 reference	
Interleukin-10-1082						
A/A	52/49	1.3 (0.3-6.1)	0.766	35/101	3.2 (0.3-30.2)	0.304
G/A	46/37	1.6 (0.3-7.8)	0.551	16/83	1.8 (0.2-17.1)	0.617
G/G	3/4	1.0 reference		1/7	1.0 reference	
Interleukin-10-592						
A/A	11/12	0.7 (0.3-1.7)	0.406	10/23	3.1 (1.2-8.2)	0.022
C/A	34/31	0.9 (0.5-1.6)	0.668	26/65	3.2 (1.5-6.8)	0.002
C/C	56/47	1.0 reference		16/103	1.0 reference	

-: Unable to compute. Pos: Positive; Neg: Negative.

Table 4 Patients with discordant *H pylori vacA* and *babA2* genes from antrum and corpus biopsy specimens in the same stomach

Patient	Gene	Antrum	Corpus	Diagnosis	GCIR group
1	*vacA*	*s2/m1*	*s1b/m1*	AG	A (High GCIR)
2		*s1b/m1*	*s2/m2*	NAG	A
3		*s1b/m1*	*s2/m2*	AG	A
4		*s1b/m1*	*s2/m2*	AG + IM	A
5		*s1b/m1*	*s1b/m2*	AG + IMA	A
6		*s1b/m1*	*s2/m2*	AG	A
7		*s1b/m1*	*s1b/m2*	AG + IM	B (Low GCIR)
8	*babA2*	Pos	Neg	NAG	A
9		Neg	Pos	AG + IM	A

Table 5 Statistical analysis for the prevalence of *H pylori* genes or alleles in Costa Rican clinical isolates from groups A and B

Gene/allele	Group A (*n* = 41, %)	Group B (*n* = 35, %)	OR (95% CI)	*P*-value
cagA	36 (87.8)	23 (65.7)	3.9 (1.2-12.9)	0.026
vacA s1b	36 (87.8)	24 (68.6)	3.6 (1.1-12.1)	0.041
vacA m1	33 (80.5)	22 (62.9)	2.7 (0.9-8.0)	0.068
babA2	19 (46.3)	15 (42.9)	1.1 (0.4-2.8)	0.812

Table 6 Adjusted odd ratios with 95% confidence intervals and *P*-value for combinations of host and bacterial factors according to gastric cancer in Costa Rican *H pylori*-positive patients

Combination of factors	Gastric Cancer		
	Pos/Neg	OR (95% CI)	*P*-value
IL-1β+3954_T/C, IL-1RN*2/L, IL-10-592_C/A			
Pos	10/8	4.7 (1.7-13.0)	0.002
Neg	42/183		
IL-1β+3954_T/C, IL-1RN*2/L, IL-10-592_C/A, vacA s1b/m1			
Pos	9/2	7.2 (1.4-36.4)	0.017
Neg	40/74		
IL-1β+3954_T/C, IL-1RN*2/L, vacA s1b/m1			
Pos	18/4	9.8 (2.9-32.9)	< 0.001
Neg	31/72		
IL-1RN*2/L, IL-10-592_C/A, vacA s1b/m1			
Pos	14/9	3.0 (1.1-8.1)	0.028
Neg	35/67		
IL-1β+3954_T/C, IL-10-592_C/A, vacA s1b/m1			
Pos	21/9	4.7 (1.9-11.9)	0.001
Neg	28/67		

H pylori infection[18,20,21]. In particular, IL-1β is important in initiating and amplifying the inflammatory response to *H pylori* infection, resulting in severe inflammation possibly leading to atrophic and metaplastic changes in the gastric mucosa. An association between cytokine polymorphisms in *IL-1β* and *IL-1RN*, and gastric premalignant lesions was previously reported in a Costa Rican population[33], while carriers of IL-1β+3954_T/C and IL-1RN*2/L had an increased risk for developing gastric cancer in another Costa Rican study[34]. Likewise, our results showed that the prevalence of the proinflammatory genotypes IL-1β+3954_T/C and IL-1RN*2/L was significantly higher in gastric cancer cases than in non-cancer cases, supporting the association of polymorphisms within *IL-1β* and *IL-1RN* and gastric cancer in the Costa Rican population. Our results also showed that the carriage of IL-10-592_A/A or IL-10-592_C/A was also associated with an increased risk for gastric cancer, which has been reported previously[21]. This is the first time that polymorphisms within the cytokine gene *IL-10* have been associated with increased risk for gastric cancer in a Costa Rican population. Collectively, these

studies thus suggest that in Costa Rica, the proinflammatory cytokine genetic profile of the host is involved in the development of gastric malignancy; but, it does not seem to play a main role in GCIR variability between regions.

The evaluation of *H pylori* virulence factors revealed that all *H pylori* strains detected in gastric atrophy and/or intestinal metaplasia cases were positive for *cagA*, *vacA s1b* and *vacA m1*, supporting the association of *H pylori cagA* and *vacA* genotype distribution with gastric cancer and premalignant lesions reported in a previous Costa Rican study[29]. In addition, the prevalence of *H pylori cagA* and *vacA s1b* was significantly higher in the high GCIR group than in the low GCIR group, and a tendency for an association between *vacA m1* and GCIR variation was also detected, confirming the association between *H pylori* virulence factors, specifically *cagA*, and the GCIR variability in Costa Rican regions suggested in a previous study[24]. However, additional factors, especially not yet determined host and/or environmental and lifestyle factors could also be involved in GCIR variability in Costa Rica, as it seems unlikely that this phenomenon could be solely explained by the status of *H pylori* infection. The association between several cytokine polymorphisms and gastric cancer reported in this and past Costa Rican studies may support this possibility. Furthermore, this study also showed that carriers of IL-1β+3954_T/C, IL-1RN*2/L and IL-10-592_C/A and carriers of these polymorphisms together with the presence of *H pylori vacA s1b/m1* increased the risk of gastric cancer when compared with patients not carrying any of these factors, suggesting that a synergistic effect of a combination of bacterial and host genotypes may determine the severity of the gastritis and the final outcome of *H pylori* infection. Such a suggestion has been documented in previous studies[18,20,21].

A comparative analysis of the status of the *H pylori* genes in each clinical isolate between antrum and corpus specimens demonstrated that a mixed strain infection (discordant *vacA* genes in the same stomach) was observed in six patients from the high GCIR group, but in only one patient from the low GCIR group. Likewise, the prevalence of gastric premalignant lesions, including gastric atrophy and intestinal metaplasia, was found more frequently in the high GCIR group than in the low GCIR group. The reason for the contrasting prevalence of mixed strain infection and premalignant lesions between high and low GCIR regions is still unknown. One may speculate that during persistent infection by *H pylori* due to yet undetermined factors associated with high population density areas such as urban lifestyle stress or inadequate intake of nutrients, subjects from high GCIR regions develop more severe gastric mucosal injury with atrophic and metaplastic changes, leading to a high genetic diversity of the bacterium for adaptation to this harsh gastric microenvironment. In fact, in strains isolated from Costa Rican patients, a high frequency of recombinated *H pylori* genes (ten of ten strains) has been reported[35]. Alternatively, it does not exclude the possibility that the contrasting prevalence is caused by the difference in the frequency rate of superinfection by *H pylori* strains, which according to population density or yet undetermined factors, may occur more frequently in subjects from high GCIR regions, supposing a higher possibility of infection with the more virulent strains, which in fact have been linked with the development of gastric premalignant lesions. However, such development of premalignant changes and superinfection or genetic recombination within *H pylori* remains unclear as to which is cause and which is effect. Further investigation is essential to understand this issue, especially an investigation which includes an increased number of mixed strain infection-positive cases.

To summarize, our results demonstrated that although the carriage of proinflammatory IL-1β+3954_T/C, IL-1RN*2/L, IL-10-592_C/A and IL-10-592_A/A polymorphisms was associated with an increased risk for the development of gastric cancer, the characteristics of *H pylori* infection, in particular the status of *cagA* and *vacA* genotype distribution, seemed to play a major role in gastric cancer incidence rate variability in Costa Rican regions.

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COMMENTS

Background

Costa Rica has one of the highest age-adjusted incidence and mortality rates for gastric cancer. Costa Rica has regions with contrasting gastric cancer incidence rates (GCIR). The cause of gastric cancer is thought to be multifactorial. The risk is high in subjects infected with *Helicobacter pylori* (*H pylori*) and even higher in those infected with strains co-expressing the *cagA*, *vacA s1* and *babA2* genes. Cytokine gene polymorphisms of the host, IL-1β, IL-1RN and IL-10, in response to *H pylori* infection, have also been associated with an increased risk for developing gastric cancer.

Research frontiers

The research in this area is focused on the evaluation of host genetic factors such as interleukin (IL)-1β (-511 and +3954), IL-10 (-1082 and -592) and IL-1RN intron 2 variable number of tandem repeat (VNTR) polymorphisms in response to *H pylori* infection, and *H pylori cagA*, *vacA* and *babA2* genotype distribution on the association with the GCIR variability in Costa Rica. A total of 191 *H pylori*-positive patients were enrolled for the determination of cytokine gene polymorphisms. Clinical isolates from gastric specimens of 83 patients were used for the PCR-based genotyping of the *H pylori cagA*, *vacA* and *babA2* genes.

Innovations and breakthroughs

Cytokine polymorphisms showed no association with GCIR variability. However, gastric atrophy, intestinal metaplasia and strains with different *vacA* genotypes in the same stomach (mixed strain infection) were more frequently found in the high GC risk group than in the low GC risk group, and *cagA* and *vacA s1b* were significantly associated with high GCIR (P = 0.026 and 0.041, respectively). IL-1β+3954_T/C (OR 2.1, 1.0-4.3), IL-1RN*2/L (OR 3.5, 1.7-7.3) and IL-10-592_C/A (OR 3.2, 1.5-6.8) were individually associated with GC, and a combination of these cytokine polymorphisms with *H pylori vacA s1b* and *m1* further increased the risk (OR 7.2, 1.4-36.4).

Applications

Although a proinflammatory cytokine genetic profile showed an increased risk for developing GC, the characteristics of *H pylori* infection, in particular the status of *cagA* and *vacA* genotype distribution seem to play a major role in GCIR variability in Costa Rica.

Peer review

This study revealed that bacterial factors (i.e., *cagA* and *vacA*, but not *babA2*) are involved in regional differences in gastric cancer risk in Costa Rica, although host factors (IL-1B, IL-1RN and IL-10 polymorphisms) are associated individually with gastric cancer risk. There are interesting points found in this study in Costa Rica, where gastric risk and genetic distribution on *H pylori* are uniquely heterogeneous.

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