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Capsaicin consumption, *Helicobacter pylori* CagA status and *IL1B-31C* > *T* genotypes: A host and environment interaction in gastric cancer

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Abstract

Gastric cancer (GC) has been associated with a complex combination of genetic and environmental factors. In contrast to most countries, available information on GC mortality trends showed a gradual increase in Mexico. Our aim was to explore potential interactions among dietary (chili pepper consumption), infectious (*Helicobacter pylori*) and genetic factors (*IL1B-31* genotypes) on GC risk. The study was performed in three areas of Mexico, with different GC mortality rates. We included 158 GC patients and 317 clinical controls. Consumption of capsaicin (Cap), the pungent active substance of chili peppers, was estimated by food frequency questionnaire. *H. pylori* CagA status was assessed by ELISA, and *IL1B-31* genotypes were determined by TaqMan assays and Pyrosequencing in DNA samples. Multivariate unconditional logistic regression was used to estimate potential interactions. Moderate to high Cap consumption synergistically increased GC risk in genetically susceptible individuals (*IL1B-31C* allele carriers) infected with the more virulent *H. pylori* (CagA+) strains. The combined presence of these factors might explain the absence of a decreasing trend for GC in Mexico. However, further research on gene–environment interactions is required to fully understand the factors determining GC patterns in susceptible populations, with the aim of recommending preventive measures for high risk individuals.

Keywords

Gastric cancer; Capsaicin; Chili pepper; Interleukin 1 beta polymorphism; *Helicobacter pylori* CagA+

Conflict of Interest

The authors declare that there are no conflict of interest.

1. Introduction

Gastric cancer (GC) is the world's 5th most frequent cancer, is still the second most common cause of cancer-related death in the world, and is thought to be associated with a complex combination of genetic and environmental factors. In contrast to most countries, available information on the GC mortality trends showed a gradual increase in Mexico (Tovar-Guzman et al., 2001). Previous epidemiological studies have independently reported an increased GC risk due to chili pepper consumption (Lopez-Carrillo et al., 2003; Sipetic et al., 2003; Mathew et al., 2000), to *Helicobacter pylori* (*H. pylori*) CagA seropositivity (Eslick, 2006; Huang et al., 2003), or to interleukin 1 beta (*IL1B*) genotypes (*IL1B-31C > T*) (Kumar et al., 2009; Vincenzi et al., 2008; Lee et al., 2007b; Wang et al., 2007; Camargo et al., 2006; Kamangar et al., 2006).

Chili pepper consumption has been related to an increased risk of GC in studies performed in Spain (Goiriena de Gandarias et al., 1988), Serbia (Sipetic et al., 2003), Korea (Lee et al., 1995), and India (Mathew et al., 2000; Gajalakshmi and Shanta, 1996). Similar adjusted results have been obtained in México where high consumption of chili is also customary (Lopez-Carrillo et al., 1994, 2003). Capsaicin (Cap) is the pungent active substance of chili peppers, and experimental evidence suggests that Cap ingestion significantly increases the number of lymphocytes and produces exfoliation of the intestinal epithelium (Johnson, 2007; Diaz Barriga et al., 1995). Several studies using diverse initiation–promotion protocols have shown that chili extract has tumor promoting effects in rodent stomach, liver and colon (Johnson, 2007). In addition, Marques et al. (2002) reported that Cap (10–200 µM) significantly induced micronuclei and sister chromatid exchanges in cultured peripheral human blood lymphocytes. Both reports illustrated the carcinogenic and genotoxic properties of Cap.

H. pylori infection is considered a necessary but not sufficient cause of gastric adenocarcinoma (IARC, 1994), because less than 3% of infected patients develop this neoplasia (Uemura et al., 2001). *H. pylori* strain virulence, host genetic characteristics and variations in dietary patterns are considered concomitant risk factors for GC development (World Cancer Research Fund/American Institute for Cancer Research, 2007; Stewart and Kleihues, 2003; Gonzalez et al., 2002). The key pathophysiological event in *H. pylori* infection is the initiation of an inflammatory response (Hatakeyama, 2006). *IL1B* is a proinflammatory host gene up-regulated by *H. pylori* infection encoding IL1β, the cytokine which initiates and amplifies the inflammatory response to *H. pylori* infection, besides being the most powerful acid inhibitor known. Moreover, virulent *H. pylori* strains, such as those bearing the Cag pathogenicity island, produce more aggressive mucosal damage (El Omar et al., 2000).

Several *IL1B* gene polymorphisms, including *IL1B-31C > T* (*IL1B-31*), have been associated with increased expression of IL1β (Vilaichone et al., 2005; Hwang et al., 2002; Pociot et al., 1992). Inconsistent epidemiological evidence has related those single nucleotide polymorphisms (SNPs) with GC risk in Caucasian and Asian populations (Wang et al., 2007; Camargo et al., 2006; Kamangar et al., 2006). However, limited information is available for Mexican populations. Only two studies have been published and both reported an increased risk for GC among carriers of the *IL1B-31C* allele (Sicinschi et al., 2006; Garza-Gonzalez et al., 2005).

Our research group has previously evaluated the interaction between Cap consumption and *H. pylori* antibodies on GC risk and showed no significant interaction between these factors, probably because the virulence of *H. pylori* was not considered (Lopez-Carrillo et al., 2003). We later examined the interaction between *H. pylori* CagA positive infections and host

IL1B-31 genotypes and showed a significantly adjusted increased risk of intestinal-type GC (OR 3.19; 95% CI 51.05–9.68) in CagA positive subjects with the *IL1B-31CC* genotype (Sicinschi et al., 2006). Therefore, our aim was to explore a potential interaction among dietary (Cap consumption), infectious (*H. pylori*) and genetic factors (*IL1B-31C* genotypes) on GC risk.

2. Materials and methods

2.1. Study population

In this report, we included 158 patients with GC and 317 clinical controls, who had serum samples available for DNA extraction and who had participated in a hospital-based case-control study performed in three geographical areas of Mexico, with low (Mexico City), medium (Puebla) and high (Yucatan) GC mortality rates (Lopez-Carrillo et al., 2003). In the original study (234 cases and 468 controls), undertaken between 1994 and 1996, we were able to identify approximately 75% of all new GC patients recorded by the Mexico National Cancer Registry during the period of recruitment in the study area from government hospitals.

2.1.1. Cases—All GC cases were histologically confirmed as adenocarcinoma of the stomach (with no other history of cancer), and classified by a single experienced cancer pathologist according to Lauren's criteria (Lauren, 1965) as follows: 82 diffuse, 57 intestinal, and 19 mixed. From these 158 cases, 55 were from hospitals in Yucatan (34.8%), 38 patients were from Puebla (24.1%) and 65 patients from Mexico City (41.1%). Information regarding the anatomical site of tumor origin was not available.

2.1.2. Controls—At least two hospital controls were matched to each case by age (± 5 years), sex, and city of residence. Inclusion criteria were as follows: no previous or current cancer, and absence of mental and immunosuppressive disorders. Individuals with evidence for past or present peptic ulcer disease, gastritis, cirrhosis, and diabetes mellitus were excluded. The most frequent diagnoses among controls ($n = 317$) were circulatory system disorders (18.3%); diseases of the nervous system and sensory organs, excepting psychiatric syndromes (15.5%); osteo-muscular and connective tissue disorders (11.0%); injuries and poisoning (10.4%); diseases of the respiratory tract (10.1%); diseases of the genitourinary system (10.4%) and the skin (7.3%); other subjects were healthy individuals attending the hospitals for preventive purposes such as vaccination or Pap smear (10.1%), and 6.9% had other illnesses that included infectious diseases, endocrine and metabolic disorders, complications of delivery and postpartum, or congenital anomalies.

Each subject provided informed consent before entering the study. The ethics committee of Mexico National Institute of Public Health approved the original study.

2.2. Capsaicin consumption

A detailed description of the method for estimating Cap consumption is described elsewhere (Lopez-Carrillo et al., 2003). Briefly, we used a validated semi-quantitative food questionnaire that comprised 133 food items in Mexico City (including 20 types of chili peppers and 6 dishes prepared with chili); 134 items in Puebla (including 14 types of chili peppers and 7 dishes prepared with chili); and 147 items in Yucatán (including 14 types of chili peppers and 3 dishes prepared with chili). The types of chilies and dishes prepared that were included in the questionnaire, were those more frequently consumed and were well known for each study area and were not necessarily the same in the three areas.

The frequency of consumption of pre-defined portions of chili peppers and dishes prepared with chilies reported by the subjects was further multiplied by its corresponding Cap content. The total individual amount of Cap consumption was estimated by adding up the Cap content for the daily reported consumption of each chili and chili-dish (Lopez-Carrillo et al., 2003).

2.3. *H. pylori* CagA status

H. pylori CagA status was determined by the presence of IgG antibodies against CagA protein in serum. Those antibodies were measured by a previously validated ELISA (97% sensitivity and 83% specificity). The cut-off value for this assay was 1.5 absorbance units (Camorlinga-Ponce et al., 1998).

2.4. Genotyping

DNA was isolated from 140 µl of serum using the Qiagen QIAmp Viral RNA Mini kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, following the vacuum method. DNA was quantitated using the Picogreen Assay (Molecular Probes, Eugene, OR). Whole blood samples were not available for this purpose. Allele discrimination for *IL1B-31* was performed using the 5' Nuclease (TaqMan) assay and by Pyrosequencing. The Taqman assay was designed by the Assays-by-Design™ service (Applied Biosystems, Foster City, CA) and performed according to standardized conditions recommended by the manufacturer. Amplification primers were (forward) CCCCTTTCCTTTAACTTGATTGTGAAATC and (reverse) AGGTTTGGTATCTGCCAGTTTCTC. Alleles were distinguished by probes labeled with different dyes, as follows: VIC—CTGTTTTTATGGCTTTCA—NFQ for the C allele and FAM—CTGTTTTTATAGCTTTCA—NFQ for the T allele. If signals for both dyes were present, the individual was considered heterozygous.

PCR amplification was performed in a reaction consisting of 1–20 ng of genomic DNA, 1× TaqMan Universal Master Mix (Applied Biosystems) and 1× Assay Mix, containing 250 nM of each probe, and 900 nM of each primer. The amplification protocol was 10 min at 95 °C, followed by 60 cycles consisting of 92 °C for 15 s and 60 °C for 1 min. Amplification and sequence detection were performed using an ABI PRISM™ 7700 instrument, with Sequencing Detection System software (SDS 1.9.1).

The Pyrosequencing assay was designed using the SNP Assay Design Software version 1.0 (Biotage, Uppsala, Sweden). Primer sequences were (forward) TTTCTCAGCCTCCTACTTCTGC, (reverse) GTGCCTCGAAGAGGTTTGGTAT, and (sequencing) CCCTCGCTGTTTTTAT. PCR amplifications were performed with 1–15 ng of DNA from each serum sample, using 1.0 U/µl of HotStar Taq DNA polymerase (Qiagen, St. Louis, MO), 0.2 µM of the forward and reverse primers, and 0.125 mM of dNTP. The amplification protocol was 95 °C for 15 min, followed by 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min for 49 cycles, with a final extension of 72 °C for 10 min.

Biotinylated PCR products were captured using streptavidin-coated Sepharose beads (Streptavidin Sepharose HP, Amersham Biosciences), denatured to remove the non-biotinylated strand and hybridized to the sequencing primer, following the manufacturer's specifications (Biotage/Qiagen). Pyrosequencing was performed using a PSQ HS 96 instrument, and genotyping calls were made by the PSQ HS 96A Software Version 1.2. In a small number of cases in which the genotype differed between TaqMan and Pyrosequencing assays, conventional sequencing was used to establish the genotype.

2.5. Statistical analysis

Selected sociodemographic characteristics, *IL1B-31* genotype frequencies, Cap consumption and *H. pylori* CagA status were compared between cases and controls using Chi-square or student-*t* tests, as appropriate. Genotype and allele frequencies in the controls (overall and by geographic area) were tested for departures from Hardy–Weinberg equilibrium (HWE). Cap consumption was grouped in two categories due to the small sample size: the first category included individuals with low consumption (<30 mg/day, approximately equivalent to the Cap content in less than three jalapeño peppers/day), whereas the other represented moderate and high consumption, (ranging from 30 to 250 mg/day, equivalent to 3–25 jalapeño peppers/day).

The association between *IL1B-31* genotypes and GC risk according to *H. pylori* CagA status and Cap consumption was evaluated by unconditional logistic regression models adjusting by age, sex, years of schooling, geographic area, smoking and methylenetetrahydrofolate reductase polymorphism (*MTHFR* 677C> T), that was associated with GC risk in this population (Lacasana-Navarro et al., 2006). The multiplicative terms between Cap consumption (moderate-high/low), *H. pylori* CagA status (positive/negative) and/or *IL1B-31* (C/T alleles) were introduced in separate models to determine the statistical significance of the Wald χ^2 test for the effect modification term (interaction). The Stata program version 9.0 was used to perform all statistical analyses (StataCorp LP, College Station, TX).

3. Results

3.1. General characteristics

The characteristics of study subjects are presented in Table 1. The proportion of individuals with a CagA seropositive status was significantly ($p = 0.007$) higher in cases (79.8%) than in controls (67.8%). By design, the distribution of age, sex, and geographic area were not significantly different between cases and controls. Other variables showing no significant crude differences between groups included genotype frequencies of *IL1B-31*, Cap consumption and smoking. In the total sample of controls, the genotype frequencies for *IL1B-31* did not depart significantly from those expected under HWE, but a slight deficiency of heterozygotes was observed in the control group from Yucatan (data not shown).

3.2. Capsaicin, *H. pylori*, and *IL1B-31* on gastric cancer risk

A significantly increased GC risk (OR = 3.41, IC₉₅ = 1.12–10.43) was observed among individuals with moderate to high Cap consumption, who were both *H. pylori* CagA positive and *IL1B-31C* allele carriers, as compared to T carriers (Table 2). Cap consumption and *IL1B-31C* alleles synergistically increased GC risk (p for interaction = 0.04) but Cap did not interact with *H. pylori* CagA status. No association was found among moderate to high Cap consumers who were *H. pylori* CagA seronegative. No associations with GC risk were found among low Cap consumers not with standing their CagA or *IL1B-31* genotype status. Non-significant interaction terms were further estimated between *H. pylori* CagA status and *IL1B-31* genotypes with and without Cap consumption (Data not shown).

4. Discussion

The main findings of our study were that moderate to high Cap consumption synergistically increases the risk of GC in genetically susceptible individuals (*IL1B-31C* allele carriers) infected with the more virulent *H. pylori* (CagA positive) strains.

Although dietary, infectious and genetic factors may interact to increase GC risk, little information is available to explain their potential mechanisms of action and/or interaction. An increased inflammatory response and an altered gastric acidic environment may be a common pathway for Cap consumption, *H. pylori* infection, and *IL1B-31* genotypes in gastric carcinogenesis. For example, subchronic and chronic Cap administration has been shown to increase gastrointestinal inflammatory response in experimental animals. Erosion of the mucosal layer of the gastrointestinal tract has been documented in Wistar rats fed Cap (0.5%, 1%, 2% and 5%) for 4–8 weeks (Srinivasan et al., 1980) whereas rats fed with a diet containing 10% of the dried chili pepper fruit for 8 weeks showed exfoliation of the intestinal epithelium into the lumen with lymphocytic accumulation (al Qarawi and Adam, 1999). In contrast, the acute administration of Cap (160 μ M) has been reported to protect against aspirin-induced injury in the rat perfused stomach assay (Holzer et al., 1989) and also in humans administered with 20 g of chili followed by aspirin (600 mg) and assessed 6 h later (Yeoh et al., 1995). A related widely studied mechanism is the inflammatory action of *H. pylori* on the gastric mucosa, since it is likely that the chronic inflammatory process triggered by the presence of the bacteria on the gastric mucosa contributes to an environment conducive to neoplastic transformation given that many of the mediators and byproducts of inflammation are mitogenic and mutagenic (Lochhead and El Omar, 2007). In contrast, short term *in vitro* studies have reported that *H. pylori* CagA+ growth was inhibited by Cap (50–500 μ g/ml) (Jones et al., 1997), whereas Lee et al. (2007a) reported that non-toxic doses of Cap (100 μ mol/L) inhibited *H. pylori* (Vac A+, CagA+ wild-type)-induced interleukin-8 (IL-8) production by gastric epithelial cells (AGS or MKN45) by modulating the I κ B, NF- κ B, and IL-8 pathways, and suggested that the acute Cap administration has potential anti-inflammatory properties (Lee et al., 2007a). The results mentioned above suggest that Cap differentially affects the processes of inflammation and GC carcinogenesis depending on the dose and duration of exposure. The low doses and short term Cap exposures that have been used in most experimental studies contrast with the higher Cap daily consumptions (0.4–3.6 mg/kg/day) estimated from individuals classified in the moderate/high consumption category (~3.5 jalapeño peppers per day by a 70 kg adult) and longer periods of exposure estimated in our study.

Another mechanism by which Cap could indirectly increase GC risk is the induction of a less acidic gastric environment favoring *H. pylori* growth, since experimental studies have shown that Cap alters gastrin secretion, the main hormone regulating gastric acid secretion. Imatake et al. (2009) reported that Cap administration (50, 100, or 500 mg/ml) to lumen-perfused stomachs of Wistar rats inhibited the acid secretion resulting from vagal stimulation without significant effects on basal acid output (Imatake et al., 2009). Regarding studies performed on humans, Mozsik et al. (1999) reported that intragastric administration of low Cap doses (100–800 μ g) to healthy human subjects reduced in a dose-dependent manner the volume of gastric juice and acid output (Mozsik et al., 1999). More recently, Ericson et al. (2009) showed that a chili-rich diet (1.4–4.2 mg Cap per day) consumed for 3-weeks by human volunteers decreased *ex-vivo* the secretion of gastrin by a suspension of antral glands obtained by gastric biopsies, whereas an increased secretion was observed when a single Cap dose was added *in vitro* to the isolated glands (Ericson et al., 2009).

The increased GC risk observed in *IL1B-31C* allele carriers infected with *H. pylori* CagA+ in this study was consistent with earlier studies showing a relationship between CagA seropositivity and GC (Eslick, 2006; Huang et al., 2003) and with previous reports showing a threefold increased GC risk in Chinese patients infected with *H. pylori* and carrying *IL1B-511TT*, as compared with CC carriers (Li et al., 2007). *IL1B-511T* is in near complete linkage disequilibrium with the *IL1B-31C* SNP (Rad et al., 2003; El Omar et al., 2000). These findings have been explained by the up-regulation of the proinflammatory cytokine IL1 β , which in turn has been reported to play an important role in the inflammatory

response and subsequent hypochlorhydria observed during chronic *H. pylori* infection, since it has been suggested that hosts carrying *IL1B-511T/T* and suffering *H. pylori* infection have increased IL1 β production (Rad et al., 2003; El Omar et al., 2000).

Among the limitations of our study are that clinical controls may not adequately represent the prevalence of the independent variables in the general population, since some of them may be related to their diseases. However, regarding chili pepper intake, our estimates in clinical controls were congruent with data on *per capita* chili pepper consumption (~30 g/day) reported in Mexico (SIAP, 2012). In addition, the prevalence of CagA+ *H. pylori* in our controls (67.8%) was in good agreement with that reported (61.6%) for the Mexican population in a national survey (Torres et al., 1998). Moreover, the genotype distribution of *IL1B* did not depart from the expected values according to the Hardy Weinberg Equilibrium test. Hence, it is unlikely that the prevalence of the studied factors differed significantly from that of the target population. Another limitation was the use of a food frequency questionnaire to estimate chili pepper consumption, because recall bias may have been present. However, we have found that chili pepper consumption was not significantly related with beliefs about health outcomes among Mexicans (Lopez-Carrillo et al., 1995), and in the present investigation interviewers were blind to the study hypothesis. In this context, while the possibility of differential recall of chili pepper intake is low, a non-differential error that biased the results towards the null value should not be disregarded. Concerning *H. pylori*, it is known that many GC patients may have previously suffered severe atrophic gastritis and metaplasia, conditions contributing to decrease *H. pylori* colonization; therefore, near the date of diagnoses there may be an increase of false negative seropositivity to *H. pylori* among cases (Forman et al., 1994). This differential measurement error could have also biased our results towards the null value.

In Mexico, about 30% of the population are high consumers of chili pepper (Lopez-Carrillo et al., 2003), and about 62% are *H. pylori* CagA+ (Torres et al., 1998). Furthermore, the risk-associated *IL1B-31C* allele has been reported to have a frequency of 65% in our studies in central and southern Mexico (Sicinski et al., 2006) and of 56% in northern Mexico (Garza-Gonzalez et al., 2005). This combination would increase the likelihood that the more virulent CagA+ *H. pylori* strains could elicit a more aggressive inflammatory response particularly among *IL1B-31C* carriers, therefore increasing GC risk. The combined presence of these factors might explain the absence of a decreasing trend in GC incidence in Mexico. However, further research on gene–environment interactions is required to fully understand the potential combination of factors determining GC patterns in the more susceptible populations, with the purpose of identification of preventive dietary measures for high risk populations.

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Abbreviations

GC	gastric cancer
Cap	capsaicin
ELISA	enzyme-linked immunosorbent assay
ILB1	interleukin 1 beta

SNPs	single nucleotide polymorphisms
HWE	Hardy-Weinberg equilibrium
MTHFR	methylenetetrahydrofolate reductase

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Table 1

General characteristics of the study population.

Characteristics	Controls (<i>n</i> = 317)	Cases (<i>n</i> = 158)	<i>p</i> Value ^{<i>a</i>}
Age in years, Mean ± SD	58.63 ± 12.55	58.59 ± 12.13	0.97
Male, <i>n</i> (%)	184 (58.04)	85 (53.80)	0.38
<i>Geographic area, n (%)</i>			
Yucatan	111 (35.02)	55 (34.81)	0.43
Mexico City	114 (35.96)	65 (41.14)	
Puebla	92 (29.02)	38 (24.05)	
Schooling years, Mean ± SD	4.42 ± 4.15	4.38 ± 4.24	0.93
Current smokers, <i>n</i> (%)	143 (45.11)	68 (43.04)	0.67
<i>H. pylori</i> CagA positive, <i>n</i> (%)	215 (67.82)	126 (79.75)	<0.01
<i>IL1B-31, n (%)</i> ^{<i>b</i>}			
TT	49 (15.46)	25 (15.82)	0.91
TC	121 (38.17)	57 (36.08)	
CC	147 (46.37)	76 (48.10)	
C carrier	415 (65.46)	209 (66.14)	0.84
<i>Capsaicin consumption in mg/day, n (%)</i>			
Low (<30)	201 (63.41)	94 (59.49)	0.41
Moderate/high (30–250)	116 (36.59)	64 (40.51)	

^{*a*} *p* Values for difference from chi-square or *t* test.^{*b*} *p* < 0.05 For HWE test among all controls.

Table 2

Interaction between capsaicin consumption, *H. pylori* CagA status and *IL1B-31* genotypes on gastric cancer risk.^a

Genotypes of <i>IL1B-31</i>		Capsaicin consumption			
		Low (<30 mg/day)		Moderate/high (30–250 mg/day)	
		CagA negative		CagA positive	
		Controls/ Cases	OR (95% CI)	Controls/ Cases	OR (95% CI)
TT		5/3	1.00	15/12	1.00
TC		31/4	0.16 (0.02–1.62)	53/30	0.78 (0.31–1.98)
CC		30/9	0.69 (0.08–5.72)	67/36	0.67 (0.27–1.63)
C carrier		61/13	0.39 (0.05–2.88)	120/66	0.71 (0.30–1.67)
<i>p</i> for multiplicative interaction		Cap consumption × <i>H. pylori</i> CagA status: 0.18 ^b			
		Cap consumption × <i>IL1B-31</i> C carrier: 0.04 ^c			

^a Adjusted by age, sex, geographic location, years of schooling, smoking, and *MTHFR* 677C>T polymorphism.^b Adjusted as in footnote a plus *ILB-31C* carrier.^c Adjusted as in footnote a plus *H. pylori* CagA status.