World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v20.i25.8151

World J Gastroenterol 2014 July 7; 20(25): 8151-8157
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE CONTROL STUDY

Interactions between pork consumption, CagA status and *IL-1B-31* genotypes in gastric cancer

Xiao-Qin Wang, Paul D Terry, Li Cheng, Hong Yan, Jian-Sheng Wang, Wen-An Wu, Sen-Ke Hu

Xiao-Qin Wang, Hong Yan, Department of Statistics and Epidemiology, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Paul D Terry, Departments of Public Health and Surgery, University of Tennessee, Knoxville, TN 37996, United States
Li Cheng, Department of Nursing, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Jian-Sheng Wang, Department of Thoracic Oncosurgery, First Affiliated Hospital of Medical School, Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Wen-An Wu, Department of Radiation Oncology Cancer Hospital of Shaanxi Province, Xi'an 710061, Shaanxi Province, China
Sen-Ke Hu, Lab Center of Public Health Department, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, Shaanxi Province, China
Author contributions: Wang XQ and Hu SK performed the majority of experiments; Wang JS and Cheng L provided vital reagents and analytical tools and were also involved in editing the manuscript; Terry PD revised the manuscript for important intellectual content and also were involved in designing the study; Wu WA and Wang JS collected all the human materials; Yan H and Wang XQ provided financial support for this work; Wang XQ designed the study and wrote the manuscript.
Supported by Grant of Health Department of Shaanxi Province, No. 2009K12-02
Correspondence to: Xiao-Qin Wang, Associate Professor, Department of Statistics and Epidemiology, School of Medicine, Xi'an Jiaotong University, 76 West Yanta Road, Xi'an 710061, Shaanxi Province, China. wangxiaoqin@mail.xjtu.edu.cn
Telephone: +86-29-82657015 Fax: +86-29-82657015
Received: December 25, 2013 Revised: February 9, 2014
Accepted: April 2, 2014
Published online: July 7, 2014

Abstract

AIM: To explore potential interactions among *Helicobacter pylori* (*H. pylori*), CagA status, interleukin *(IL)-1B-31* genotypes, and non-cardiac gastric cancer (GC) risk.

METHODS: A case-control study of non-cardia GC was performed at 3 hospitals located in Xi'an, China, between September 2008 and July 2010. We included 171 patients with histologically diagnosed primary non-cardia GC and 367 population based controls (matched by sex, age and city of residence). A standardized questionnaire was used to obtain information regarding potential risk factors, including pork consumption. *H. pylori CagA* status was assessed by enzyme-linked immunosorbent assay, and *IL-1B-31* genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism. Multivariate unconditional logistic regression was used to explore potential interactions among the factors.

RESULTS: The *CagA* appeared to confer an increased risk of GC (OR = 1.81, 95%CI: 1.25-2.61). The main associations with *IL-1B-31C* allele here were 0.98 (95%CI: 0.59-1.63) for CC *vs* TT and 0.99 (95%CI: 0.64-1.51) for C Carriers *vs* TT. However, no associations were observed for *CagA* or *IL-1B-31* genotype status among subjects who reported low pork consumption (*P* for interaction = 0.11). In contrast, high pork consumption and *IL-1B-31C* genotypes appeared to synergistically increase GC risk (*P* for interaction = 0.048) after adjusting for confounding factors, particularly among subjects with *CagA* (OR = 3.07, 95%CI: 1.17-10.79). We did not observe effect modification of pork consumption by *H. pylori CagA* status, or between *H. pylori CagA* status and *IL-1B-31* genotypes after adjustment for pork consumption and other factors.

CONCLUSION: These interaction relationships among *CagA*, *IL-1B-31* and pork consumption may have implications for development of the preventive strategies for the early detection of non-cardiac GC.

Key words: Gastric cancer; Pork; *CagA*; interleukin-*1B*; Interaction; *Helicobacter pylori*

Core tip: It is widely known that infectious, dietary, and

genetic factors are implicated in gastric carcinogenesis, which is a long, complicated, and multi-stage process. The *Helicobacter pylori* (*H. pylori*) virulence factor *CagA* has been shown to be polymorphic and to contribute to disease pathogenesis in an allele-dependent manner. The interleukin *(IL)-1* gene plays an important role in determining the long-term outcome of *H. pylori* infection. Dietary factors such as pork consumption may contribute to the malignancy process in synergy with these genetic factors and infectious agents. Our study further explores potential interactions among dietary (pork intake), infectious (*H. pylori CagA* positive) and genetic factors (*IL-1B-31* genotypes) on gastric cancer risk.

Wang XQ, Terry PD, Cheng L, Yan H, Wang JS, Wu WA, Hu SK. Interactions between pork consumption, CagA status and *IL-1B-31* genotypes in gastric cancer. *World J Gastroenterol* 2014; 20(25): 8151-8157 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i25/8151.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i25.8151

INTRODUCTION

Gastric cancer (GC) is the second leading cause of cancer-related mortality in the world. It is widely known that infectious, dietary, and genetic factors are implicated in gastric carcinogenesis, which is a long, complicated, and multi-stage process[1]. GC is strongly associated with *Helicobacter pylori* (*H. pylori*) infection; however, most infected persons never develop this malignancy. The *H. pylori* virulence factors *CagA* and *VacA* have each been shown to be polymorphic and to contribute to disease pathogenesis in an allele-dependent manner[2]. The most studied of these is *CagA* effector protein[3], a 120e 145-kDa protein[4], which is located at the end of an approximately 40-kb cluster of genes called cag pathogenicity island (PAI). Cag PAI encodes a type-Ⅳ secretion system and transfers *CagA* protein into host cells[5]. Upon entering the host cells, *CagA* can trigger IL-8 secretion, thereby priming an inflammatory response[6,7] and promoting cell proliferation, scattering and migration through phosphorylation-dependent and independent mechanisms[5].

The interleukin *(IL)-1* gene plays an important role in determining the long-term outcome of *H. pylori* infection[8]. It contains three related genes, *IL-1A, IL-1B*, and *IL-1RN*, which encode the pro-inflammatory cytokines IL-1a and IL-1b[9]. IL-1b regulates the expression of several genes involved in inflammation. It is encoded by a 7.5 kb gene, and the expression is regulated by both distal and proximal promoter elements[10,11]. The polymorphisms of *IL-1B-31T/C* in the promoter region of the gene have been intensively studied[12]. The first published report showed a positive association between GC and the *IL-1B-31C* allele[13], which has been confirmed in subsequent studies[14,15].

The consumption of red meat and processed meat has risen in developed and developing countries, which may have implications for GC occurrence[16-18]. Pork is the major red meat consumed by people in China[19]. Some previous studies have found positive associations between the consumption of pork and GC risk[20,21], whereas others have not[22-24]. Five studies were included in a meta-analysis in 2013, and the summary relative risk of the association between pork and GC risk was 1.31 (95%CI: 0.97-1.78)[25]. Hence, a positive association has been suggested, but remains inconclusive.

Several interactions have been noted among these variables. For example, *H. pylori* infected individuals with the *IL-1B-31CC* genotype tend to secrete less *IL-1B* and appear to be more susceptible to precancerous lesions[26]. Perhaps noteworthy, a statistically significant interaction was found between *IL-1B-31* and *CagA* status for the risk of intestinal-type GC in a Mexican population[27]. Furthermore, red meat intake was found to interact with *H. pylori* infection in the development of GC in the EPIC study[28], which showed that red meat intake was associated with an increased risk of non-cardia gastric cancer, particularly in *H. pylori* antibody-positive subjects. In contrast, our previous case-control study found that red meat intake did not interact with *H. pylori* infection in the process of gastric carcinogenesis[29], possibly because specific host genetic factors, such as *IL-1B-31*, were not considered. Therefore, our present study aimed to explore potential interactions among *H. pylori* status, *IL-1B-31C* genotypes, pork consumption and GC risk.

MATERIALS AND METHODS

Ethics

This study was approved by the Ethics Committee of the School of Medicine, Xi'an Jiaotong University. All patients provided informed written consent.

Study population

We included 171 patients with non-cardia GC and 367 population-based controls who had serum samples available for DNA extraction. The original study included 257 cases and 514 controls, and was undertaken between September 2008 and July 2010[29]. All cases were aged 30 to 79 years and had pathologically confirmed non-cardia GC. Patients with other major chronic diseases, including other forms of cancer (particularly diseases affecting dietary patterns or communication), were excluded. After identification, eligible patients or their family members were invited to sign consent forms and participate in the study. Two population-based controls were matched to each case by age (± 5 years), sex, and city of residence. The control subjects were confirmed to be free of cancer, diabetes, and gastrointestinal disorders.

Pork consumption

We measured the pork consumption of study participants using a Food Frequency Questionnaire[30]. Participants were asked about the average frequencies and

portion sizes of 121 food items consumed during the preceding year, including the type of pork dishes that were typically consumed in the study region. If dietary changes had occurred during the past year, information regarding dietary habits prior to the change was elicited.

The quantity of each food item was represented by a Chinese food weight unit, Liang (equivalent to 50 g), for most investigated food items. Food consumption frequency was ranked in 9 categories: from "never or less than 1 time per month" to "2 or more times per day." Food items were grouped based on the China Food Composition 2004 classification proposed by the Chinese Center for Disease Control[31]. We previously validated the food frequency questionnaire using a 24-h diet record[30]. For pork consumption, the Pearson correlation coefficients of the validity and reproducibility of the food frequency questionnaire were 0.49 and 0.58, respectively.

Other measured variables

Several non-dietary variables were assessed through the use of a general questionnaire. This questionnaire included items regarding personal and family medical history, medications used, physical activity (number of hours of sedentary activities, and light, moderate, or heavy physical activities), alcohol consumption (number of alcoholic beverages per week), smoking (age at commencement and smoking intensity), and lifestyle factors (*e.g.*, vitamin supplement intake, refrigerator use).

H. pylori CagA status

The antibody to *H. pylori* was tested with an enzyme-linked immunosorbent assay kit (Human HP-Ap enzyme-linked immunosorbent assay Kit, San Diego, CA). A finding of at least 10 units per milliliter in the blood was considered to indicate the presence of antibody against *H pylori*. *CagA*-positive *H. pylori* infection was defined as the presence of *CagA* antibody in the serum.

Genotyping

The primer was designed with Primer Premier 5 software and synthesized by the Invitrogen Company (*ILB-31* forward, GAAGCTTCCACCAATACTC and reverse, AGCACCTAGTTGTAAGGAAG). Genotyping for *IL-1B-31 (T/C)* polymorphisms was performed by means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). It was performed in a 50 μL PCR mixture containing 10 × buffer 5 μL, $MgCl_2$ 23 μL, dNTP 3 μL, upstream and downstream primers 1 μL, respectively, 1.25U DNA polymerase, DNA 50 ng, with sterile distilled water added to 50 μL. Thermal cycling conditions were 94 ℃ for 5 min 45 s; 35 cycles of 94 ℃ for 45 s, 56 ℃ for 45 s, and 72 ℃ for 45 s, and 72 ℃ for 5min. PCR products were digested by Alu I restriction enzyme (the mixture: PCR product 10 μL, Alu I 10 U, 10 × buffer Tango TM 3 μL, with sterile distilled water added to 30 μL, followed by incubation at 37 ℃ overnight). The genotype was determined by agarose gel electrophoresis.

Statistical analysis

For selected sociodemographic characteristics, *IL-1B-31* genotype frequencies, pork consumption and *H. pylori CagA* status, comparisons between cases and controls were made using *t* tests and χ^2 tests. The association between *IL-1B-31* genotypes and GC risk according to *H. pylori CagA* status and pork consumption was evaluated using unconditional logistic regression models with adjustment for age, gender, education, smoking, alcohol, and family history. To estimate the combined effects of pork consumption, *H. pylori CagA* status, and *IL-1B-31* genotypes, pork consumption was separated in two categories according to the mean distribution of the control group, the low (< 25 g/d) and high consumption categories (> 25 g/d). The multiplicative terms between pork consumption (high/low), *H. pylori CagA* status (positive/negative) and/or *IL-1B-31* (C/T alleles) were introduced in separate models to determine the statistical significance of the Wald χ^2 test for the interaction term. SPSS software version 17.0 (IBM, Armonk, NY) was used to perform all statistical analyses.

RESULTS

The characteristics of study subjects are presented in Table 1. The distributions of gender and education levels were not significantly different between cases and controls. The proportion of individuals with seropositive status was higher in cases (59.06%) than in controls (44.41%) (P = 0.002). The main association with *Cag A* here was 1.81 (95%CI: 1.25-2.61). There were no significant crude differences between groups based on pork consumption and genotype frequencies of *IL-1B-31*. The *IL-1B-31C* allele didn't appeared to confer an increased risk of GC (OR = 0.98, 95%CI: 0.59-1.63 CC *vs* TT; OR = 0.99, 95%CI: 0.64-1.51 C carriers *vs* TT for main association with IL-1B-31C here). In the total sample of controls, the genotype frequencies for *IL-1B-31* did not depart from those expected under Hardy-Weinberg equilibrium[32].

The *IL-1B-31C* allele appeared to confer an increased risk, particularly among *CagA*-positive subjects with high pork consumption (OR = 3.07, 95%CI: 1.17-10.79) (Table 2). Pork consumption and *IL-1B-31C* alleles synergistically increased GC risk (P for interaction = 0.048), whereas pork consumption did not show interaction with *H. pylori CagA* status (P for interaction = 0.11). No association was found among high pork consumers who were *H. pylori CagA* seronegative. Furthermore, no associations with GC risk were found among low pork consumers based on their *CagA* or *IL-1B-31* genotype status. In multivariate models that adjusted for pork consumption and other factors, we did not observe statistically significant interaction between *H. pylori CagA* status

Table 1 Demographic data of cases and controls *n* (%)

Characteristic		Cases *n* = 171	Controls *n* = 367	t/χ^2	*P* value
Age (yr, mean ± SD)		56.93 ± 14.01	56.81 ± 13.90	0.093[1]	0.440
Gender					
	Male	118 (69.01)	243 (66.21)	0.412	0.521
	Female	53 (30.99)	124 (33.79)		
Education					
	Primary	51 (29.82)	117 (31.88)	0.332	0.847
	Secondary	84 (49.12)	179 (48.77)		
	Tertiary and postgraduate	36 (21.06)	71 (19.35)		
BMI (kg/m^2)					
	≤ 25	110 (64.33)	244 (66.49)	0.365	0.794
	> 25	61 (35.67)	123 (33.51)		
H. pylori CagA positive		101 (59.06)	163 (44.41)	10.018	0.002[a]
IL-1B-31					
	TT	41 (23.98)	89 (24.25)	0.005	0.997
	TC	84 (49.12)	180 (49.05)		
	CC	46 (26.90)	98 (26.70)		
	C carrier	130 (76.02)	278 (75.75)		
Pork consumption					
	< 25 g/d	89 (52.05)	177 (48.23)	0.680	0.410
	≥ 25 g/d	82 (47.95)	190 (51.77)		

[1]For *t* test. [a]*P* < 0.05 *vs* control group. BMI: Body mass index; *H. pylori*: *Helicobacter pylori*; IL: Interleukin.

Table 2 Joint effects of pork consumption, *CagA* status and interleukin-*1B-31* genotypes on the risk of gastric cancer[1]

Genotype of *IL-1B-31*	Pork consumption							
	Low (< 25 g/d)				High (≥ 25 g/d)			
	Hp CagA (-)		Hp CagA (+)		Hp CagA (-)		Hp CagA (+)	
	Case/control	OR	Case/control	OR	Case/control	OR	Case/control	OR
TT	9/15	1.00	16/15	1.00	11/30	1.00	5/29	1.00
TC	11/58	0.42 (0.14-1.11)	30/39	0.71 (0.31-1.98)	20/49	1.25 (0.47-2.81)	23/34	2.98 (0.99-11.30)
CC	12/27	0.71 (0.27-2.36)	11/23	0.46 (0.16-1.54)	27/25	0.86 (0.29-2.35)	16/23	3.11 (1.08-12.66)
C carrier	23/85	0.45 (0.18-1.37)	41/62	0.69 (0.33-1.63)	27/74	1.00 (0.48-2.06)	39/57	3.07 (1.17-10.79)

[1]Adjusted for the following confounding factors: age, gender, education, smoking, alcohol, and family history. *P* for multiplicative interaction: Pork consumption and *Helicobacter pylori* (*H. pylori*) *CagA* status: 0.11 [adjusted by age, gender, education, smoking, alcohol, family history and interleukin *(IL)-1B-31 C* carrier]; Pork consumption and *IL-1B-31 C* carrier: 0.048 (adjusted by age, gender, education, smoking, alcohol, family history and *H. pylori CagA* status).

and *IL-1B-31* genotypes.

DISCUSSION

In the present study, we observed an increased GC risk among individuals with high pork consumption, particularly among subjects who were both *H. pylori* (*CagA*) positive and genetically susceptible (*IL-1B-31C*) allele carriers. If further studies confirm that *CagA, IL-1B-31* and high pork consumption interact in the development of GC, this would have implications for cancer prevention in China, a country with notably high rates of GC.

Regarding a possible interaction between *IL-1B-31* genotype and *CagA* status, our present study showed a marginally significant interaction term for the risk of GC (*P* for interaction = 0.078), a finding we consider interesting in light of the results of three previously epidemiological studies. Charkravorty's study showed that *H. pylori*-infected individuals with the *IL-1B-31CC* genotype secrete less *IL-1B* and may have increased susceptibility to precancerous lesions[26]. Rad's study found that carriers of the proinflammatory *IL-1B-511T/-31C* and *IL-1RN2* alleles had an increased risk for the development of intestinal metaplasia, atrophic gastritis (AG), and severe inflammation, with ORs of 1.7 (95%CI: 0.8-3.4) to 4.4 (95%CI: 1.5-12.9)[33]. Liviu's study found a statistically significant interaction between *IL-1B-31* and *CagA* status for the risk of intestinal-type GC (*P* = 0.023)[27]. It was hypothesized that some GCs may be the outcome of a synergy between effects of the *IL-1B-31C* carrier and the *CagA* positive *H. pylori* microorganisms, which can induce and amplify the inflammatory response, and thereby cause *IL-1B* secretion and hypochlorhydria[27].

As the major virulence factor of *H. pylori, CagA* disturbs cellular functions by physically interacting with and deregulating intracellular signaling molecules *via* both tyrosine phosphorylation dependent and independent mechanisms after delivery into gastric epithelial cells[34]. Once translocated into host cytoplasm, *CagA* may bind to the inner surface of the cell membrane and undergo

tyrosine phosphorylation[35]. The phosphorylated and unphosphorylated forms of *CagA* interact with a number of host proteins to activate downstream signal pathways, such as inducing ornithine decarboxylase upregulation *via* Src/MEK/ERK/c-Myc pathway[36] and directing REG3γ expression in gastric epithelial cells *via* activation of the *IL-11*/gp130/STAT3 pathway[37]. Non-phosphorylated *CagA* may activate the hepatocyte growth factor/scatter factor receptor c-Met and adaptor protein Grb2, induce phosphorylation of phospholipase C gamma and impair the *E-cadherin/b-catenin* complex formation, and mediate the inhibition of the kinase partitioning-defective 1b/microtubule affinity-regulating kinase 2 (PAR1b/ MARK2) to perturb atypical protein kinase C signaling[35]. In a recent experiment[38], transgenic zebrafish expressing either the wild-type or a phosphorylation-resistant form of *CagA* exhibited significantly increased rates of intestinal epithelial cell proliferation and showed significant upregulation of the Wnt target genes cyclinD1, axin2 and the zebrafish c-mycorthologmyca. Additionally, *CagA* was shown to induce higher levels of IL-8 production, activate nuclear factor κB (NF-κB), AP-1 and FAT[7], and enhance the activity of transforming growth factor-β-activated kinase 1 (TAK1) and TAK1-induced NF-κB activation *via* the TRAF6-mediated K63-linked ubiquitination of TAK1, which in turn is used by *CagA* for the *H. pylori* induced inflammatory response[39]. This might also inhibit miR-370 expression, which may lead to overexpression of FoxM1 and consequent increased intestinal cell proliferation[40]. These findings suggest multiple roles of *CagA* in gastric carcinogenesis.

Our study has several limitations. Given our case-control study design, information regarding past pork consumption may have been misclassified to some extent. To reduce misclassification of dietary exposures, we designed and validated our questionnaire using the 24-h diet record method[30]. The results showed that the questionnaire had reasonable validity and reliability. Nonetheless, the misclassification of diet remains a potential source of bias in our data. Another limitation is the potential misclassification of *H. pylori* infection status. In the present study, *H. pylori* was detected after non-cardia GC was diagnosed; hence, infection may not have been present in all subjects as premalignant lesions progressed[41]. This type of misclassification would tend to attenuate the association between *H pylori* infection and non-cardia GC. Additionally, the sample size of the present study was not optimal for the analysis of the potential interactions among pork intake, infectious (*CagA*) and genetic factors (*IL-1B-31C* genotypes) on GC risk. Therefore, it is possible that some of our modeled interaction terms did not reach statistical significance due to insufficient sample size.

In summary, we found statistically significant interactions among *CagA*, *IL-1B-31* and high pork consumption in their association with non-cardiac GC. In China, people consume much more pork than beef and lamb, and the majority of individuals are *H. pylori CagA* positive. These findings may have implications for primary prevention, and also secondary preventive measures aimed at the early detection of GC in China. For example, a greater understanding of how common exposures interact in GC etiology may lead to selective screening of high-risk individuals based, at least in part, on levels of those interacting risk factors.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the generous assistance of the staff members of each participating hospital.

COMMENTS

Background

It is widely known that infectious, dietary, and genetic factors are implicated in gastric carcinogenesis, which is a long, complicated, and multi-stage process. Thus, gastric cancer might be caused by potential interactions among dietary (pork intake), infectious and genetic factors.

Research frontiers

The *Helicobacter pylori* (*H. pylori*) virulence factor *CagA* has been shown to be polymorphic and to contribute to disease in an allele-dependent manner. The interleukin *(IL)-1* gene plays an important role in determining the long-term outcome of *H. pylori* infection. Dietary factors such as pork consumption may contribute to the malignancy process in synergy with these genetic factors and infectious agents.

Innovations and breakthroughs

The study further explores potential interactions among dietary (pork intake), infectious (*H. pylori CagA* positive) and genetic factors (*IL-1B-31C* genotypes) on gastric cancer (GC) risk.

Applications

These findings may have implications for preventive measures aimed at the early detection of GC in China. For example, a greater understanding of how common exposures interact in GC etiology may lead to selective screening of high-risk individuals based, at least in part, on levels of those interacting risk factors.

Terminology

CagA effector protein, a 120e 145-kDa protein, is located at the end of an approximately 40-kb cluster of genes called cag pathogenicity island. The *IL-1* gene contains three related genes, *IL-1A, IL-1B,* and *IL-1RN*, which encode the pro-inflammatory cytokines IL-1a and IL-1b. *IL-1b* regulates the expression of several genes involved in inflammation. Both *CagA* and *IL-1B-31* may play a modifying role in the association between pork and GC risk.

Peer review

This is a good case-control study in which authors explored the interactions among dietary (pork intake), infectious (*H. pylori)* and genetic factors (*IL-1B-31C* genotypes). These findings may have implications for preventive measures aimed at the early detection of GC in China. The results are interesting and may represent multi-factor interaction mechanism of gastric carcinogenesis.

REFERENCES

1 **Kuo SH**, Chen LT, Lin CW, Wu MS, Hsu PN, Tsai HJ, Chu CY, Tzeng YS, Wang HP, Yeh KH, Cheng AL. Detection of the Helicobacter pylori CagA protein in gastric mucosa-associated lymphoid tissue lymphoma cells: clinical and biological significance. *Blood Cancer J* 2013; **3**: e125 [PMID: 23852160 DOI: 10.1038/bcj.2013.22]

2 **Bridge DR**, Merrell DS. Polymorphism in the Helicobacter pylori CagA and VacA toxins and disease. *Gut Microbes* 2013; **4**: 101-117 [PMID: 23380646 DOI: 10.4161/gmic.23797]

3 **Wandler AM**, Guillemin K. Transgenic expression of the Helicobacter pylori virulence factor CagA promotes apoptosis or tumorigenesis through JNK activation in Drosophila.

PLoS Pathog 2012; **8**: e1002939 [PMID: 23093933 DOI: 10.1371/journal.ppat.1002939]
4 **Covacci A**, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N. Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993; **90**: 5791-5795 [PMID: 8516329]
5 **Backert S**, Selbach M. Role of type IV secretion in Helicobacter pylori pathogenesis. *Cell Microbiol* 2008; **10**: 1573-1581 [PMID: 18410539 DOI: 10.1111/j.1462-5822.2008.01156.x]
6 **Wei GC**, Chen J, Liu AY, Zhang M, Liu XJ, Liu D, Xu J, Liu BR, Ling H, Wu HX, DU YJ. Prevalence of Helicobacter pylori vacA, cagA and iceA genotypes and correlation with clinical outcome. *Exp Ther Med* 2012; **4**: 1039-1044 [PMID: 23226771]
7 **Backert S**, Naumann M. What a disorder: proinflammatory signaling pathways induced by Helicobacter pylori. *Trends Microbiol* 2010; **18**: 479-486 [PMID: 20863705 DOI: 10.1016/j.tim.2010.08.003]
8 **Palli D**, Saieva C, Luzzi I, Masala G, Topa S, Sera F, Gemma S, Zanna I, D'Errico M, Zini E, Guidotti S, Valeri A, Fabbrucci P, Moretti R, Testai E, del Giudice G, Ottini L, Matullo G, Dogliotti E, Gomez-Miguel MJ. Interleukin-1 gene polymorphisms and gastric cancer risk in a high-risk Italian population. *Am J Gastroenterol* 2005; **100**: 1941-1948 [PMID: 16128937]
9 **Dinarello CA**. Biologic basis for interleukin-1 in disease. *Blood* 1996; **87**: 2095-2147 [PMID: 8630372]
10 **Shirakawa F**, Saito K, Bonagura CA, Galson DL, Fenton MJ, Webb AC, Auron PE. The human prointerleukin 1 beta gene requires DNA sequences both proximal and distal to the transcription start site for tissue-specific induction. *Mol Cell Biol* 1993; **13**: 1332-1344 [PMID: 8441379]
11 **Monks BG**, Martell BA, Buras JA, Fenton MJ. An upstream protein interacts with a distinct protein that binds to the cap site of the human interleukin 1 beta gene. *Mol Immunol* 1994; **31**: 139-151 [PMID: 8309477]
12 **Lind H**, Haugen A, Zienolddiny S. Differential binding of proteins to the IL1B -31 T/C polymorphism in lung epithelial cells. *Cytokine* 2007; **38**: 43-48 [PMID: 17587593]
13 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398-402 [PMID: 10746728]
14 **Machado JC**, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, Castro Alves C, Campos ML, Van Doorn LJ, Caldas C, Seruca R, Carneiro F, Sobrinho-Simões M. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. *Gastroenterology* 2003; **125**: 364-371 [PMID: 12891537]
15 **Sicinschi LA**, Lopez-Carrillo L, Camargo MC, Correa P, Sierra RA, Henry RR, Chen J, Zabaleta J, Piazuelo MB, Schneider BG. Gastric cancer risk in a Mexican population: role of Helicobacter pylori CagA positive infection and polymorphisms in interleukin-1 and -10 genes. *Int J Cancer* 2006; **118**: 649-657 [PMID: 16114018]
16 **Daniel CR**, Cross AJ, Koebnick C, Sinha R. Trends in meat consumption in the USA. *Public Health Nutr* 2011; **14**: 575-583 [PMID: 21070685 DOI: 10.1017/S1368980010002077]
17 **Zamani N**, Hajifaraji M, Fazel-tabar Malekshah A, Keshtkar AA, Esmaillzadeh A, Malekzadeh R. A case-control study of the relationship between gastric cancer and meat consumption in Iran. *Arch Iran Med* 2013; **16**: 324-329 [PMID: 23725064]
18 **Aune D**, De Stefani E, Ronco A, Boffetta P, Deneo-Pellegrini H, Acosta G, Mendilaharsu M. Meat consumption and cancer risk: a case-control study in Uruguay. *Asian Pac J Cancer Prev* 2009; **10**: 429-436 [PMID: 19640186]
19 **Yu M**, Gao Q, Wang Y, Zhang W, Li L, Wang Y, Dai Y. Unbalanced omega-6/omega-3 ratio in red meat products in China. *J Biomed Res* 2013; **27**: 366-371 [PMID: 24086169 DOI: 10.7555/JBR.27.20130066]
20 **Ito LS**, Inoue M, Tajima K, Yamamura Y, Kodera Y, Hirose K, Takezaki T, Hamajima N, Kuroishi T, Tominaga S. Dietary factors and the risk of gastric cancer among Japanese women: a comparison between the differentiated and non-differentiated subtypes. *Ann Epidemiol* 2003; **13**: 24-31 [PMID: 12547482]
21 **Phukan RK**, Narain K, Zomawia E, Hazarika NC, Mahanta J. Dietary habits and stomach cancer in Mizoram, India. *J Gastroenterol* 2006; **41**: 418-424 [PMID: 16799882]
22 **You WC**, Blot WJ, Chang YS, Ershow AG, Yang ZT, An Q, Henderson B, Xu GW, Fraumeni JF, Wang TG. Diet and high risk of stomach cancer in Shandong, China. *Cancer Res* 1988; **48**: 3518-3523 [PMID: 3370645]
23 **Kim HJ**, Chang WK, Kim MK, Lee SS, Choi BY. Dietary factors and gastric cancer in Korea: a case-control study. *Int J Cancer* 2002; **97**: 531-535 [PMID: 11802218]
24 **Tokui N**, Yoshimura T, Fujino Y, Mizoue T, Hoshiyama Y, Yatsuya H, Sakata K, Kondo T, Kikuchi S, Toyoshima H, Hayakawa N, Kubo T, Tamakoshi A. Dietary habits and stomach cancer risk in the JACC Study. *J Epidemiol* 2005; **15** Suppl 2: S98-108 [PMID: 16127240]
25 **Zhu H**, Yang X, Zhang C, Zhu C, Tao G, Zhao L, Tang S, Shu Z, Cai J, Dai S, Qin Q, Xu L, Cheng H, Sun X. Red and processed meat intake is associated with higher gastric cancer risk: a meta-analysis of epidemiological observational studies. *PLoS One* 2013; **8**: e70955 [PMID: 23967140 DOI: 10.1371/journal.pone.0070955]
26 **Chakravorty M**, Ghosh A, Choudhury A, Santra A, Hembrum J, Roychoudhury S. Interaction between IL1B gene promoter polymorphisms in determining susceptibility to Helicobacter pylori associated duodenal ulcer. *Hum Mutat* 2006; **27**: 411-419 [PMID: 16550552]
27 **Queiroz DM**, Guerra JB, Rocha GA, Rocha AM, Santos A, De Oliveira AG, Cabral MM, Nogueira AM, De Oliveira CA. IL1B and IL1RN polymorphic genes and Helicobacter pylori cagA strains decrease the risk of reflux esophagitis. *Gastroenterology* 2004; **127**: 73-79 [PMID: 15236174]
28 **González CA**, Jakszyn P, Pera G, Agudo A, Bingham S, Palli D, Ferrari P, Boeing H, del Giudice G, Plebani M, Carneiro F, Nesi G, Berrino F, Sacerdote C, Tumino R, Panico S, Berglund G, Simán H, Nyrén O, Hallmans G, Martinez C, Dorronsoro M, Barricarte A, Navarro C, Quirós JR, Allen N, Key TJ, Day NE, Linseisen J, Nagel G, Bergmann MM, Overvad K, Jensen MK, Tjonneland A, Olsen A, Bueno-de-Mesquita HB, Ocke M, Peeters PH, Numans ME, Clavel-Chapelon F, Boutron-Ruault MC, Trichopoulou A, Psaltopoulou T, Roukos D, Lund E, Hemon B, Kaaks R, Norat T, Riboli E. Meat intake and risk of stomach and esophageal adenocarcinoma within the European Prospective Investigation Into Cancer and Nutrition (EPIC). *J Natl Cancer Inst* 2006; **98**: 345-354 [PMID: 16507831]
29 **Wang XQ**, Yan H, Terry PD, Wang JS, Cheng L, Wu WA, Hu SK. Interaction between dietary factors and Helicobacter pylori infection in noncardia gastric cancer: a population-based case-control study in China. *J Am Coll Nutr* 2012; **31**: 375-384 [PMID: 23529995]
30 **Wang X**, Sa R, Yan H. Validity and reproducibility of a food frequency questionnaire designed for residents in north China. *Asia Pac J Clin Nutr* 2008; **17**: 629-634 [PMID: 19114401]
31 **Yang Y**, Wang G, Pan X. "Food Composition Table of China 2004." Beijing: Beijing University Press, 2004: 5-150
32 **Sham P**. Statistics in human genetics. London: Arnold Publishers, 2001: 50-79
33 **Rad R**, Prinz C, Neu B, Neuhofer M, Zeitner M, Voland P, Becker I, Schepp W, Gerhard M. Synergistic effect of Heli-

cobacter pylori virulence factors and interleukin-1 polymorphisms for the development of severe histological changes in the gastric mucosa. *J Infect Dis* 2003; **188**: 272-281 [PMID: 12854083]

34 **Liu Z**, Xu X, Chen L, Li W, Sun Y, Zeng J, Yu H, Chen C, Jia J. Helicobacter pylori CagA inhibits the expression of Runx3 via Src/MEK/ERK and p38 MAPK pathways in gastric epithelial cell. *J Cell Biochem* 2012; **113**: 1080-1086 [PMID: 22266963 DOI: 10.1002/jcb.23440]

35 Available from: URL: http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation/fresh-pork-from-farm-to-table/CT-Index

36 **Xu X**, Liu Z, Fang M, Yu H, Liang X, Li X, Liu X, Chen C, Jia J. Helicobacter pylori CagA induces ornithine decarboxylase upregulation via Src/MEK/ERK/c-Myc pathway: implication for progression of gastric diseases. *Exp Biol Med* (Maywood) 2012; **237**: 435-441 [PMID: 22442341 DOI: 10.1258/ebm.2011.011199]

37 **Lee KS**, Kalantzis A, Jackson CB, O'Connor L, Murata-Kamiya N, Hatakeyama M, Judd LM, Giraud AS, Menheniott TR. Helicobacter pylori CagA triggers expression of the bactericidal lectin REG3γ via gastric STAT3 activation. *PLoS One* 2012; **7**: e30786 [PMID: 22312430 DOI: 10.1371/journal.pone.0030786]

38 **Neal JT**, Peterson TS, Kent ML, Guillemin K. H. pylori virulence factor CagA increases intestinal cell proliferation by Wnt pathway activation in a transgenic zebrafish model. *Dis Model Mech* 2013; **6**: 802-810 [PMID: 23471915 DOI: 10.1242/dmm.011163]

39 **Lamb A**, Yang XD, Tsang YH, Li JD, Higashi H, Hatakeyama M, Peek RM, Blanke SR, Chen LF. Helicobacter pylori CagA activates NF-kappaB by targeting TAK1 for TRAF6-mediated Lys 63 ubiquitination. *EMBO Rep* 2009; **10**: 1242-1249 [PMID: 19820695 DOI: 10.1038/embor.2009.210]

40 **Feng Y**, Wang L, Zeng J, Shen L, Liang X, Yu H, Liu S, Liu Z, Sun Y, Li W, Chen C, Jia J. FoxM1 is overexpressed in Helicobacter pylori-induced gastric carcinogenesis and is negatively regulated by miR-370. *Mol Cancer Res* 2013; **11**: 834-844 [PMID: 23576572 DOI: 10.1158/1541-7786.MCR-13-0007]

41 **Muñoz N**, Kato I, Peraza S, Lopez G, Carrillo E, Ramirez H, Vivas J, Castro D, Sanchez V, Andrade O, Buiatti E, Oliver W. Prevalence of precancerous lesions of the stomach in Venezuela. *Cancer Epidemiol Biomarkers Prev* 1996; **5**: 41-46 [PMID: 8770465]

P- Reviewers: Chen XZ, Kita H
S- Editor: Gou SX **L- Editor**: Wang TQ **E- Editor**: Liu XM

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327