Short Communication

CagA-producing *Helicobacter pylori* and increased risk of gastric cancer: a nested case–control study in Korea

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In a nested-case control study of 100 cases of gastric cancer and 400 matched controls in relation to virulence factors of *Helicobacter pylori* in a Korean cohort, CagA seropositivity was significantly associated with a higher risk of gastric cancer among *H. pylori*-infected subjects (OR = 3.57, 95% CI 1.05–12.14).

Keywords: gastric cancer; *Helicobacter pylori*; CagA; Cohort study; Korea

Gastric cancer is the first major incident cancer with an age-standardized incidence rate of 69.6 in males and 26.8 in females per 100,000 in Korea, the highest in the world (Forlay et al, 2004; Shin et al, 2005b). *Helicobacter pylori* (HP) was classified as a group 1 human carcinogen for gastric cancer by the International Agency for Research on Cancer in 1994 (IARC, 1994). However, despite the evidence that HP infection increases gastric cancer risk, the prevalence of HP infection does not always correlate positively with risk (Uemura et al, 2001; Peek and Blaser, 2002; Lunet and Barros, 2003). In fact, certain Asian and African countries with a high prevalence of HP infection have a low incidence of gastric cancer (Lunet and Barros, 2003).

One explanation for the above differences concerns virulence factors, such as cytotoxin-associated antigen (CagA) and vacuolating cytotoxin (VacA), produced by HP strains, that may be more carcinogenic to the gastric epithelium (Peek and Blaser, 2002). These factors can invade epithelial cells in stomach walls and induce epithelial responses with carcinogenic potential (Peek and Crabtree, 2006).

We previously reported a null association between HP infection and gastric cancer in a nested case–control study within the Korean Multi-Center Cancer Cohort (KMCC) (Shin et al, 2005a). We have here investigated the virulence factors, CagA and/or VacA seropositivity, in relation to gastric cancer susceptibility.

**MATERIALS AND METHODS**

The Korean Multi-Center Cancer Cohort (KMCC) is a prospective cancer cohort based upon four urban or rural areas in Korea (Yoo et al, 2002). Participants over age 30 years were recruited from 1993 through 2004. A detailed standardized questionnaire on general lifestyle, physical activity, dietary habit, reproductive factors, and past medical history was completed for each subject by interviewers at the time of recruitment. Blood and urine samples were donated voluntarily. Blood samples were then stored at −70°C and urine samples at −20°C. The study protocol was approved by the Institutional Review Boards of the Seoul National University Hospital and the National Cancer Center of Korea. All subjects provided written informed consent.

As of December 2002, 136 gastric cancer cases were identified among the 14,440 cohort members through a computerized record linkage to the Korea Central Cancer Registry database and the National Health Insurance database. Of these, we excluded gastric cancer cases diagnosed before recruitment (n = 36). To validate a diagnosis of gastric cancer and to obtain additional detailed clinical information such as tumour site, a medical record review was undertaken in all such cases. For comparison, four controls from the eligible cancer-free cohort were matched to each cancer case by incidence density sampling based on age (within 5 years), gender, area of residence and the year of recruitment.

Sera were assayed using immunoblot kits (Helico Blot 2.1™, MP Biomedicals Asia Pacific, Singapore) to identify IgG antibodies specific for HP according to the manufacturer’s instruction. CagA and VacA seropositivity and HP infection status were determined using these kits. Sensitivities for HP infection, and CagA and VacA seroposivities have been reported to be 99, 99 and 93%, and specificities to be 98, 90 and 88%, respectively (Park et al, 2002).

The demographic characteristics of cases and controls were compared using the χ² test. Conditional logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). In subgroup analyses stratified by HP IgG antibody, unconditional logistic regression models were used because the matches of cases and controls were not preserved after
RESULTS

Table 1 shows the baseline characteristics of the study subjects. The mean age of cases was 63 years and two-thirds were male. Of the cases, 34% were never-smokers and 44% never-drinkers and 27% were uneducated. Smoking history and alcohol drinking history, and years of education were not significantly different between cases and controls. In all, 75 cases were non-cardiac gastric cancer and 87 were adenocarcinoma. The median interval from initial blood collection to the diagnosis of gastric cancer was 2.4 years.

Table 2 shows the OR for gastric cancer in relation to HP infection and the virulence factors. HP infection was found to be associated with gastric cancer (OR = 0.96, 95% CI 0.68–1.36). CagA and VacA seropositivity was not found to elevate the risk of gastric cancer (OR = 1.10, 95% CI 0.83–1.47; OR = 1.04, 95% CI 0.85–1.28, respectively). The risk of HP infection and CagA and VacA seropositivity were not found to be significantly different for gender (male vs female) and the period of follow-up (<2.4 vs ≥2.4 years) (data not shown).

The associations of CagA and VacA seropositivity on the risk for gastric cancer were evaluated stratified by HP IgG antibody and CagA and VacA seropositivity. The associations of CagA and VacA seropositivity were not found to be significantly different for gender (male vs female) and the period of follow-up (<2.4 vs ≥2.4 years) (data not shown).

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Table 3 Odds ratios and 95% confidence intervals for gastric cancer according to the CagA and VacA seropositivity stratified by H. pylori IgG antibody and CagA and VacA seropositivity

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between HP and CagA. CagA-positive strains have been reported as being more virulent with respect to atrophic gastritis, intestinal metaplasia and gastric cancer development (Hatakeyama, 2004). It may also be relevant that in the Mongolian gerbil, CagA-positive HP strains caused more severe inflammation in gastric mucosa than did CagA-negative strains (Dhar et al., 2003).

In a recent meta-analysis of 16 epidemiologic studies, the overall OR for CagA seropositivity among HP-infected subjects was 1.49 (95% CI 1.25 – 1.77) (Huang et al., 2003). Several studies have failed to detect a positive association between VacA protein sero-positivity and gastric cancer risk (Shimoyama et al., 1999; Yamaoka et al., 1999), although Rudi et al. (1997) reported an elevated risk of gastric cancer (OR = 1.74, 95% CI 1.08 – 2.70) in VacA seropositive participants (Rudi et al., 1997).

The risk of gastric cancer associated with CagA observed in the present study was higher than that in the meta-analysis by Huang et al. It has been suggested that the distribution and pathogenicities of HP subtypes found in East Asia differ from those found in Western countries (Hatakeyama, 2004). In Europe and the US the cagA1 subtype of the cagA gene is dominant, whereas the cagA2 subtype, which is more biologically active and virulent, is exclusively found in East Asia (Gonzalez et al., 2003). Genotypes for CagA were not investigated in the present study. Nevertheless, if the majority of HP strains infecting the Korean population are the cagA2 subtype, the higher risk found in the present study than in Western studies concurs with the putative mechanistic role of this subtype in gastric carcinogenesis.

Prospective and community-based cohort design of the present study minimized the possibility of misclassification for exposure. Using the residence registration number, which is a unique identifier for each individual in Korea, the follow-up data linkages were established and enabled complete identification of cancer development status and death.

However, the study also has certain limitations. The small number of gastric cancer patients (n = 100) and the low frequency of HP-negative subjects (10%) limits the statistical power to evaluate the effect of HP infection and virulence factors. The misclassification of exposure to HP infection due to seroreversion of HP in the elderly with gastric atrophy and the relatively short period of follow-up might have influenced our results (Kikuchi, 2002). But the risks of HP infection and virulence factors were not significantly different for the follow-up period ( <2.4 vs ≥2.4 years). The direction of its influence, if it existed, would have been toward the null. Measurement of serum pepsinogen I and II levels would have been helpful in terms of identifying participants with premalignant lesions and preventing the misclassification (Watabe et al., 2005).

Our study suggests that CagA-producing HP increases the risk of gastric cancer in the Korean population, although it should be noted that a large proportion of healthy controls are also infected with CagA- or VacA-producing HP. Some nutrients, food components and host genetic polymorphisms may be involved in gastric carcinogenesis associated with HP infection (Hamajima, 2003; Correa, 2004). Further studies on individual genetic susceptibilities and dietary habits, and on the effects of bacterial variants should be pursued.

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REFERENCES
Correa P (2004) The biological model of gastric carcinogenesis. IARC Sci Publ 157: 301 – 310
Dhar SK, Soni BK, Das BK, Mukhopadhyay G (2003) Molecular mechanism of action of major Helicobacter pylori virulence factors. Mol Cell Biochem 253: 207 – 215
Ferlay J, Bray F, Pisani P, Parkin DM (2004) GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide. IARC CancerBase No. 5. version 2.0, Vol. 2005. Lyon: IARC Press.
Gonzalez CA, Pena S, Capella G (2003) Clinical usefulness of virulence factors of Helicobacter pylori as predictors of the outcomes of infection. What is the evidence? Scand J Gastroenterol 38: 905 – 915
Hamajima N (2003) Persistent Helicobacter pylori infection and genetic polymorphisms of the host. Nagoya J Med Sci 66: 103 – 117
Hatakeyama M (2004) Oncogenic mechanisms of the Helicobacter pylori CagA protein. Nat Rev Cancer 4: 688 – 694
Huang JQ, Zheng GF, Sunanac K, Irvine EJ, Hunt RH (2003) Meta-analysis of the relationship between cagA seropositivity and gastric cancer. Gastroenterology 125: 1636 – 1644
IARC (1994) IARC Monograph on the Evaluation of Carcinogenic Risks to Humans: Schistosomes, Liver Flukes and Helicobacter Pylori, Vol. 61. Lyon: IARC.
Kikuchi S (2002) Epidemiology of Helicobacter pylori and gastric cancer. Gastric Cancer 5: 6 – 15
Lunet N, Barros H (2003) Helicobacter pylori infection and gastric cancer: facing the enigma. Int J Cancer 106: 953 – 960
Park CY, Cho YK, Kodama T, El-Zimaity HM, Osato MS, Graham DY, Yamaoka Y (2002) New serological assay for detection of putative Helicobacter pylori virulence factors. J Clin Microbiol 40: 4753 – 4756
Peek RM, Blaser MJ (2002) Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nat Rev Cancer 2: 28 – 37
Peek Jr RM, Crabtree JE (2006) Helicobacter infection and gastric neoplasia. J Pathol 208: 233 – 248
Rudi J, Kolb C, Maiwald M, Zuna L, von Herbay A, Galle PR, Stremmel W (1997) Serum antibodies against Helicobacter pylori proteins VacA and CagA are associated with increased risk for gastric adenocarcinoma. Dig Dis Sci 42: 1652 – 1659
Shin A, Shin HR, Kang D, Park SK, Kim CS, Yoo KY (2005a) A nested case-control study of the association of Helicobacter pylori infection with gastric adenocarcinoma in Korea. Br J Cancer 92: 1273 – 1275
Shin HR, Won YJ, Jung KW, Kong HJ, Yim SH, Lee JK, Noh HI, Lee JK, Pisani P, Park JC (2005b) Nationwide cancer incidence in Korea, 1999 – 2001; First resulting using the national cancer incidence database. Cancer Res Treat 37: 325 – 331
Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ (2001) New serological assay for detection of Helicobacter pylori CagA and VacA and the risk for gastric cancer. J Clin Pathol 54: 985 – 990
Watabe H, Mitsushima T, Yamaji Y, Okamoto M, Wada R, Kubo T, Doi H, Yoshida H, Kawabe T, Omata M (2005) Predicting the development of gastric cancer from combining Helicobacter pylori antibodies and serum pepsinogen status: a prospective endoscopic cohort study. Gut 54: 764 – 768
Yamaoka Y, Kodama T, Kashima K, Graham DY (1999) Antibody against Helicobacter pylori CagA and VacA and the risk for gastric cancer. J Clin Pathol 52: 215 – 218
Yoo KY, Shin HR, Chang SH, Lee KS, Park SK, Kang D, Lee DH (2002) Korean Multi-center Cancer Cohort Study including a Biological Materials Bank (KMCC-I). Asian Pac J Cancer Prev 3: 85 – 92