Helicobacter pylori (HP) infection appears protective against oesophageal adenocarcinoma (EA) risk. Matrix metalloproteinases (MMPs) are released in the presence of HP infection. In MMP2 wild-type individuals, HP was significantly protective of EA risk (adjusted odds ratio: 0.29; 95% confidence interval = 0.1 – 0.7). Matrix metalloproteinases may modulate the EA–HP relationship.

The increasing incidence of oesophageal adenocarcinoma (EA) (Younes et al, 2002) may be explained partly by the widespread nature of chronic gastro-oesophageal reflux disease (GERD) and Barrett’s oesophagus (Kim et al, 1997; Shaheen and Ransohoff, 2002). Epidemiologic studies suggest that Helicobacter pylori (HP) infection protects against EA (Chow et al, 1998; de Martel et al, 2004). A postulated mechanism for the protective effect involves acid reflux reduction with HP-mediated chronic atrophic gastritis (Sozzi et al, 1998).

A number of factors influence the chronic atrophic gastritis severity. Myeloperoxidase (MPO) released from inflammatory cells and manganese superoxide dismutase 2 (SOD2) enhance inflammation and tissue damage, thereby increasing the severity of chronic atrophic gastritis (Suzuki et al, 1995; Smoot et al, 2000). In addition, the expression of matrix metalloproteinases (MMPs), responsible for the degradation of extracellular matrix components, is increased during HP infection, thereby enhancing chronic atrophic gastritis (Bergin et al, 2004).

The Val allele of the SOD2 –Ala16Val polymorphism results in decreased SOD2 enzyme transport into mitochondria (Shimoda-Matsubayashi et al, 1996). Likewise, the A allele of MPO –463 G/A is associated with lower MPO enzyme levels (Piedrafita et al, 1996). The 2G allele of MMP1 –1607 1G/2G polymorphism is associated with higher MMP1 expression levels (Rutter et al, 1998). The T allele of MMP2 –1306C/T disrupts an Sp1 regulatory element leading to lower promoter activity and decreasing MMP2 expression (Price et al, 2001). In contrast, the MMP3 1171 –6A/5A polymorphism causes transcriptional elevation and modulates expression of MMP3 (Ye et al, 1996). MMP12 –82A G allele is associated with a higher MMP12 promoter activity (Jormsjo et al, 2000).

We hypothesise that genetic variants associated with greater inflammatory gene and higher MMP expression are associated with a greater extent of chronic atrophic gastritis and greater protection against the risk of EA.

**MATERIALS AND METHODS**

**Study population**

Local institutional review boards approved the study. All cases gave written consent, were >18 years old and were diagnosed within the last 6 months. All had histologically confirmed EA that was deemed endoscopically (or at the time of resection) to have a tumour centre located at or above the midpoint of the gastro-oesophageal junction, with at least two-thirds of the tumour bulk located in the oesophagus (Liu et al, 2007). The serum samples of 100 cases that were collected and processed in a uniform manner were analysed. They had similar age, gender and disease distribution as the 83 recruited cases that were not analysed due to serum collection problems (P > 0.10 for each comparison). A total of 101 age- and gender frequency-matched healthy controls were composed of lifetime cancer-free, GERD-free, non-blood-related family members (usually spouses) and friends of other cancer/surgical patients. For all participants, a standardised interviewer-administered questionnaire collected information on...
age, gender, race, body mass index, smoking status/history and HP infection status. Body mass index in the third decade of life was used as a surrogate of healthy adult weight decades prior to the development of EA. Over 90% of the participants were born in the United States.

Genotyping and HP determination

DNA was extracted using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). The MMP1 4167 G/A, MMP2 −1607 G/C, H. pylori infection status and CagA status were determined using the Helicoblot 2.1 kit (Genelabs Diagnostics®, Singapore City, Singapore and Redwood City, CA, USA).

For quality control determinations of laboratory techniques, positive and negative controls and blinded duplicate samples were run. Alternative genotyping approaches (different primers, endonucleases, techniques, conditions) to verify technical reliability and accuracy were used as required. A second scientist checked all laboratory interpretation independently, and a blinded third scientist arbitrated discrepancies.

Statistical analysis

Univariate and bivariate explorations of the data were performed. Case and control demographic data were compared using Fisher’s exact and Student’s t-tests, where appropriate. If homozygous variant genotype frequencies were very low, heterozygous and homozygous variant genotypes were combined. Hardy–Weinberg equilibrium was tested using the χ² test. Using standardised kit criteria, we defined four different categories of HP infection status based on the antibodies detected with the Helicoblot 2.1 kit: ever infection, current infection, infection with CagA or infection with VacA strains (Monteiro et al, 2001; Hoang et al, 2006).

Analyses of associations between the different polymorphisms, HP infection status and EA risk were based on logistic regression models (SAS, version 9.1, SAS Institute Inc., Cary, NC, USA). Where appropriate, crude and adjusted odds ratios (AOR) and 95% confidence intervals (CI) for the risk of EA were calculated. For adjusted analyses, we included adult body mass index, smoking status, age and gender. To test for a gene–HP interaction, an interaction term was added to the logistic regression model (likelihood ratio tests). The SAS macro HAPPY was used for haplotype determination and D’ calculation.

RESULTS

Baseline demographics are shown in Table 1. The recruitment rate was >85% for cases and controls. Cases had the following stages: I (n = 9), IIa (n = 18), IIb (n = 17), III (n = 22), IVa (n = 5) and IVb (n = 29). The proportions of cases and controls treated for HP infection were 91% and 12%, respectively (P > 0.30). There were no overall associations between each polymorphism and the EA risk. The overall AOR between HP infection and EA was 0.71 (95% CI = 0.41–1.0). Duplicate genotyping was performed for at least 30% of subjects for each polymorphism with 100% concordance. Serologic analysis had 99% concordance of 100% duplicates.

The strongest and most consistent association was found between the MMP2 −1306 polymorphism and HP infection: in

Table 1: Demographic information by case–control status

| Characteristics | Controls (n = 101) | Cases (n = 100) | P-value |
|-----------------|-------------------|----------------|---------|
| Gender*         |                   |                |         |
| Female          | 13 (13%)          | 12 (12%)       | 0.9     |
| Male            | 88 (87%)          | 88 (88%)       |         |
| Age (years)³   | 63 ± 8            | 64 ± 8         | 0.4     |
| Race            |                   |                |         |
| Caucasian       | 101 (100%)        | 100 (100%)     | NA      |
| Smoking status*|                   |                |         |
| Non-smokers     | 27 (27%)          | 16 (16%)       | 0.07    |
| Ex-smokers      | 54 (53%)          | 67 (67%)       |         |
| Current smoker  | 20 (20%)          | 17 (17%)       |         |
| Padyears²       | 37 ± 31           | 42 ± 36        | 0.5     |
| BMI (kg m⁻²)    |                   |                |         |
| BMI at diagnosis| 28 ± 5            | 26 ± 5         | 0.004   |
| BMI in healthy adult | 22 ± 4 | 23 ± 4 | 0.4 |
| HP infection status¹ | | | |
| HP ever infected | 43 (43%) | 36 (36%) | 0.4 |
| HP never infected | 58 (57%) | 64 (64%) | |
| CagA strains    |                   |                |         |
| Positive        | 30 (30%)          | 29 (29%)       | 0.8     |
| Negative        | 71 (70%)          | 71 (71%)       |         |
| VacA strains    |                   |                |         |
| Positive        | 20 (20%)          | 15 (15%)       | 0.4     |
| Negative        | 81 (80%)          | 85 (85%)       |         |

BMI = body mass index; HP = Helicobacter pylori; NA = not applicable. *Cases and controls were compared using Fisher’s exact tests. †Data are reported as mean (s.d.), and compared using Student’s t-tests. "Ever smokers only. "BMI in the third decade of life (twenties).

58 cases/56 controls carrying the MMP2 −1306C/C wild-type genotype, having HP infection at any time in their life was strongly protective against EA (AOR 0.29, 95% CI = 0.1–0.7). In contrast, in 35 cases/43 controls carrying MMP2 C/C or T/T (associated with a lower promoter activity), this protective effect was lost (AOR 1.76; 95% CI = 0.06–5.2; for ever infection with HP). When we specifically analysed different definitions of HP status such as current, VacA+ or CagA+ infections for their HP-EA risk association, the protective effect of HP remained significant in the wild-type genotype of MMP2 −1306C/C (AOR ranged from 0.16 to 0.35) and abrogated in patients carrying any variant allele. The corresponding interaction model and interaction term were statistically significant (P < 0.001). When using several other definitions of HP infection status, the MMP2–HP interaction terms were similarly significant: MMP2 and CagA+ infection (interaction term, P = 0.03), and MMP2 and current HP infection (interaction term, P = 0.005). Thus, both stratified analysis and interaction models point to an MMP2–HP relationship.

When evaluating other MMP polymorphisms, ever infection with HP was also associated with a significantly decreased EA risk in the subset of patients who carried the wild-type genotypes, MMP3 −1171 6A/6A (AOR 0.04, 95% CI = 0.002–0.9, P = 0.04; 27 cases/29 controls) and MMP12 −82 A/A (AOR 0.44, 95% CI = 0.2–0.8, P = 0.02; 75 cases/79 controls), but not for their corresponding variant genotypes. The MMP1 −1607, MMP3 −6A/5A and MMP12 −82A/G polymorphisms are in linkage disequilibrium (D’ = 0.5 for MMP1-MMP3; D’ = 0.8 for both MMP1-MMP12 and MMP3-MMP12), but none of the evaluated polymorphisms are linked with the MMP2 −1306 C/T polymorphism (D’ < 0.15 for all comparisons). Haplotype analyses of the MMP1-MMP3-MMP12
polymorphisms resulted in weaker associations when compared with the analyses of individual polymorphisms (data not shown).

No significant protective effects of HP infection on the EA risk were found with any genotype subsets of MPO – 463 G/A or with SOD2 Ala16Val.

DISCUSSION

To our knowledge, the question of gene – HP interaction in EA risk has not been addressed. The protective effects of HP infection on the risk of developing EA have been postulated, based on a number of epidemiologic studies (Chow et al., 1998; de Martel et al., 2005). This protection is believed to result mainly from decreased acid reflux following HP-mediated chronic atrophic gastritis (Sozzi et al., 1998). As only one in 200 individuals with Barrett’s oesophagus develop EA annually, gene – environment factors may hold the key to understanding EA risk. Yet few studies have examined gene – environment interactions in oesophageal cancer; most were conducted in Asia, evaluating squamous cell carcinoma of the oesophagus, genetic polymorphisms and either smoking or alcohol consumption (Lee et al., 2001; Yu et al., 2004; Lin et al., 2006; Hiyama et al., 2007). We compared a set of EA cases with frequency-matched controls.

The most significant finding of our study was the increased protection against the risk of developing EA by HP infection in individuals carrying the wild-type MMP2 – 1306 C/C genotype, whereas no protective effect could be detected among the variant genotypes. MMP2 is part of the MMP family, a group of enzymes responsible for the degradation of extracellular matrix components. MMP2 levels were increased in the presence of HP infection and contributed to tissue damage in HP-associated gastritis (Bergin et al., 2004). The wild-type genotype is associated with higher promoter activity and MMP2 expression (Price et al., 2001). Higher MMP2 expression may lead to increased severity of chronic atrophic gastritis and subsequently less acid reflux and decreased EA risk (Sozzi et al., 1998; Raghunath et al., 2003; Bergin et al., 2004). Our results are therefore consistent with this mechanism. This protective effect against EA risk was maintained even when different definitions of the HP infection status were evaluated. Our examination of polymorphisms of two other MMPs led to similar results.

Our results should be interpreted cautiously, given the modest sample size and multiple comparisons, although even a Bonferroni correction would still leave the primary gene – HP interaction and stratified wild-type models statistically significant. In addition, the serologic evaluation of prior HP infection over time is imperfect, and may be affected by antibiotic treatment. Nonetheless, because not just one but several MMP polymorphisms were associated independently with gene – HP interactions, this is a novel finding that warrants further exploration.

In conclusion, we were able to demonstrate for the first time a modulation of the protective effect of HP on EA risk by several polymorphisms in the MMP pathway. These intriguing results will need confirmation in a larger prospective setting, particularly one that also explores the relationships between Barrett’s oesophagus, GERD and EA prognosis.

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REFERENCES

Bergin PJ, Anders E, Sicheng W, Erik J, Jennie A, Hans L, Pierre M, Qiang PH, Marianne QJ (2004) Increased production of matrix metalloproteinases in Helicobacter pylori-associated human gastritis. Helicobacter 9: 201 – 210
Chow WH, Blaser MJ, Blot WJ, Gammon MD, Vaughan TL, Risch HA, Perez-Perez GI, Schoenberg JB, Stanford JL, Rotterdam H, West AB, Fraumeni Jr JF (1998) An inverse relation between cagA+ strains of Helicobacter pylori infection and risk of esophageal and gastric cardia adenocarcinoma. Cancer Res 58: 588 – 590
de Martel C, Llosa AE, Farr SM, Friedman GD, Vogelman JH, Orentreich N, Cotley DA, Parsonnet J (2005) Helicobacter pylori infection and the risk of development of esophageal adenocarcinoma. J Infect Dis 191: 761 – 767
Hiyama T, Yoshishara M, Tanaka S, Chayama K (2007) Genetic polymorphisms and esophageal cancer risk. Int J Cancer 121: 1643 – 1658
Hoang TT, Rehnborg AS, Wheeldon TU, Bengtsson C, Phung DC, Befrits R, Sorberg M, Granstrom M (2006) Comparison of the performance of serological kits for Helicobacter pylori infection with European and Asian study populations. Clin Microbiol Infect 12: 1112 – 1117
Jormajo S, Ye S, Moritz J, Walter DH, Dimmelser S, Zeilher AM, Henney A, Hamsten A, Eriksson P (2000) Allele-specific regulation of matrix metalloproteinase-12 gene activity is associated with coronary artery luminal dimensions in diabetic patients with manifest coronary artery disease. Circ Res 86: 998 – 1003
Kim R, Weisfeld J, Reynolds JC, Kulmer WH (1997) Etiology of Barrett’s metaplasia and esophageal adenocarcinoma. Cancer Epidemiol Biomarkers Prev 6: 369 – 377
Lee JM, Lee YC, Yang SY, Yang PW, Luh SP, Lee CJ, Chen CJ, Wu MT (2001) Genetic polymorphisms of XRCC1 and risk of the esophageal cancer. Int J Cancer 95: 240 – 246
Lin YC, Wu DC, Lee JM, Hsu HK, Kao EL, Yang CH, Wu MT (2006) The association between microsomal epoxide hydrolase genotypes and esophageal squamous-cell-carcinoma in Taiwan: interaction between areca chewing and smoking. Cancer Lett 237: 281 – 288
Liu G, Zhou W, Wang LL, Park S, Miller DP, Xu LL, Wain JC, Lynch TJ, Su L, Christiani DC (2004) MPO and SOD2 polymorphisms, gender, and the risk of non-small cell lung carcinoma. Cancer Lett 214: 69 – 79
Liu G, Zhou W, Yeap BY, Su L, Wain JC, Ponomer JS, Lynch NJ, Christiani DC (2007) XRCC1 and XPD polymorphisms and esophageal adenocarcinoma risk. Carcinogenesis 28: 1254 – 1258
Monteiro L, de Mascarel A, Sarrasqueta AM, Bergey B, Barberis C, Talpy P, Roux D, Shoulter L, Goldfain D, Lamouliatte H, Megraud F (2001) Diagnosis of Helicobacter pylori infection: noninvasive methods compared to invasive methods and evaluation of two new tests. Am J Gastroenterol 96: 353 – 358
Piedrafita FJ, Molander RB, Vansant G, Orlova EA, Pfahl M, Reynolds WF (1996) An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. J Biol Chem 271: 14412 – 14420
Price SJ, Greaves DR, Watkins H (2001) Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. J Biol Chem 276: 7549 – 7558
Raghunath A, Hungin AP, Wain JC, Childs S, Bergey B, Barberis C, Talpy P, Roux D, Shoulter L, Goldfain D, Lamouliatte H, Megraud F (2001) Diagnosis of Helicobacter pylori infection: noninvasive methods compared to invasive methods and evaluation of two new tests. Am J Gastroenterol 96: 353 – 358
Piedrafita FJ, Molander RB, Vansant G, Orlova EA, Pfahl M, Reynolds WF (1996) An Alu element in the myeloperoxidase promoter contains a composite SP1-thyroid hormone-retinoic acid response element. J Biol Chem 271: 14412 – 14420
Price SJ, Greaves DR, Watkins H (2001) Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: role of Sp1 in allele-specific transcriptional regulation. J Biol Chem 276: 7549 – 7558
Raghunath A, Hungin AP, Wosse D, Childs S (2003) Prevalence of Helicobacter pylori in patients with gastro-oesophageal reflux disease: systematic review. BMJ 326: 737
Rutter JL, Mitchell TJ, Butticc G, Meyers J, Gussella JF, Ozellus IJ, Brinkerhoff CE (1998) A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 58: 5321 – 5325
Shaheen N, Ransohoff DF (2002) Gastroesophageal reflux, Barrett esophagus, and esophageal cancer: scientific review. JAMA 287: 1972 – 1981
Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, Nakagawa-Hattori Y, Shimizu Y, Mizuno Y (1996) Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson’s disease. Biochem Biophys Res Commun 226: 561 – 565
Smoot DT, Elliott TB, Verspaget HW, Jones D, Allen CR, Vernon KG, Bremner T, Kidd LC, Kim KS, Groupman JD, Ashktorab H (2000) Influence of Helicobacter pylori on reactive oxygen-derived gastric epithelial cell injury. Carcinogenesis 21: 2091 – 2095
Sozzi M, Valentinii M, Figura N, De Paoli P, Tedeschi RM, Gloghini A, Serraino D, Poletti M, Carbone A (1998) Atrophic gastritis and intestinal metaplasia in Helicobacter pylori infection: the role of CagA status. Am J Gastroenterol 93: 375 – 379
Suzuki M, Nakamura M, Mori M, Miura S, Tsuchiya M, Ishii H (1995) Lansoprazole inhibits oxygen-derived free radical production from neutrophils activated by Helicobacter pylori. J Clin Gastroenterol 20(Suppl 2): S93 – S96
Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE, Henney AM (1996) Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J Biol Chem 271: 13055 – 13060
Younes M, Henson DE, Ertan A, Miller CC (2002) Incidence and survival trends of esophageal carcinoma in the United States: racial and gender differences by histological type. Scand J Gastroenterol 37: 1359 – 1365
Yu C, Zhou Y, Miao X, Xiong P, Tan W, Lin D (2004) Functional haplotypes in the promoter of matrix metalloproteinase-2 predict risk of the occurrence and metastasis of esophageal cancer. Cancer Res 64: 7622 – 7628