PAPERS

Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection

J Parsonnet, G D Friedman, N Orentreich, H Vogelman

Abstract

Background and aims—It is not known why some people with *Helicobacter pylori* infection develop gastric cancer whereas others do not. Whether the CagA phenotype of *H pylori* infection affected risk for cancer independently of other posited risk factors was evaluated.

Subjects—242 persons who participated in a previous nested case-control study of gastric cancer. 179 (90 cases and 89 controls) were infected with *H pylori* as determined by enzyme linked immunosorbent assay (ELISA) in serum and 63 (13 cases and 50 controls) were uninfected.

Methods—Serum samples from cases and controls, obtained a mean of 14.2 years before diagnosis of cancer in the cases, were tested by ELISA for IgG antibodies against the CagA gene product of *H pylori*. They had previously been tested for pepsinogen I. Using logistic regression analysis, risk for cancer was compared among infected persons with CagA antibodies, infected persons without CagA antibodies, and uninfected persons.

Results—Subjects infected with *H pylori* who had CagA antibodies were 5.8-fold more likely than uninfected subjects to develop gastric cancer (95% confidence interval (95% CI)=2.6–13.0). This was true for both intestinal (odds ratio (OR) 5.1, 95% CI=2.1–12.2) and diffuse type (OR 10.1, 95% CI=2.2–47.4) cancers. By contrast, *H pylori* infected subjects without CagA antibodies were only slightly, and not significantly, at increased risk for cancer (OR 2.2, 95% CI=0.9–5.4) and any possible association was restricted to diffuse type carcinoma (OR 9.0, 95% CI=1.2–65.8). Pepsinogen 1 <50 ng/ml significantly increased risk for both cancer types in *H pylori* infected persons and lessened the magnitude of association between CagA and cancer. Educational attainment, cigarette smoking, and ABO blood group were not associated with malignancy.

Conclusions—When compared with uninfected subjects, persons infected with CagA positive *H pylori* are at considerably increased risk of gastric cancer. CagA negative *H pylori* are less strongly linked to malignancy and may only be associated with diffuse type disease.

(Gut 1997; 40: 297–301)

Keywords: *Helicobacter pylori*, gastric neoplasm, CagA, pepsinogen, ABO blood group, cigarette smoking.

Infection with *Helicobacter pylori* has been identified as a risk factor for gastric adenocarcinoma of the antrum and corpus.^{1,2} Epidemiological studies indicate that infection increases risk for both intestinal and diffuse histological types of cancer between threefold and sixfold. As a consequence, the International Agency for Research on Cancer recently termed *H pylori* a group I carcinogen, a definite cause of cancer in humans.³ Yet, because only a minority of subjects infected with *H pylori* will develop cancer, other factors must be important in determining cancer occurrence. Among non-infectious risk factors, diet, cigarette smoking, and socioeconomic status have been of most enduring interest.^{4–7} Genetic characteristics of the host, such as ABO blood type, may also influence outcome of infection. Blood group O is postulated to contribute to *H pylori* adherence and to *H pylori* related duodenal ulceration.^{8,9} Blood group A, on the other hand, has been associated with the diffuse type of gastric cancer.⁷

Helicobacter pylori is not a clonal organism and exhibits great genetic diversity.^{10–13} At the phenotypic level, strains can be characterised into two types: those that contain a gene associated with cytotoxin expression, the so-called CagA gene, and those that do not.^{14,15} Expression of the CagA protein can be sensitively and specifically diagnosed by detecting antibodies to it. CagA positive *H pylori*, which comprise some 50%–60% of United States isolates, cause more extensive inflammation of the gastric mucosa than CagA negative strains.^{16–20} CagA positive infections are also more likely to progress to atrophic gastritis than CagA negative infections.²¹ Furthermore, a nested case-control study among Japanese-American men suggested that CagA antibodies are more common in infected persons with gastric malignancy than in infected persons without such malignancy.²² Whereas this finding was not statistically significant, the twofold increase in CagA positive *H pylori* among intestinal type cancers appeared unlikely to be due to chance alone.

In 1990, we conducted a nested case-control study of *H pylori* and gastric cancer among men and women in a northern California healthcare programme.¹ We found *H pylori* antibodies to

Departments of Medicine and Health Research and Policy, Stanford University School of Medicine, Stanford, California 94305, USA
J Parsonnet

Division of Research, Kaiser Permanente Care Program, Oakland, California 94611, USA
G D Friedman

Orentreich Foundation for the Advancement of Science, Cold-Spring-on-Hudson, New York 10516, USA

Correspondence to: Dr J Parsonnet, HRP Building RM T225, Stanford University, Stanford, CA 94305-5092, USA.

Accepted for publication 28 November 1996

be 3-6-fold more common in persons who later developed gastric cancer than in matched controls. Subsequently, we identified a low pepsinogen 1 level (<50 ng/ml) also to be associated with development of cancer, although only in people with *H pylori* infection.²³ Because it remains unknown why some people with *H pylori* infection develop cancer whereas others do not, we decided to assess risk factors for cancer among *H pylori* infected subjects only. Factors of interest include antibodies to the CagA protein, pepsinogen I concentration (an indicator of concurrent atrophic gastritis), ABO blood group type, and level of education (a surrogate for socioeconomic status). We also compared risk for gastric cancer among three groups: persons with CagA positive *H pylori* infection, those with CagA negative *H pylori* infection, and those without *H pylori*.

Methods

Subjects had been previously identified for a nested case-control study of *H pylori* and gastric cancer.¹ Cases of gastric cancer and controls were selected from among 128 992 subjects who had provided serum as part of a multiphasic health evaluation at Kaiser Permanente Medical Care Program between 1964 and 1969. Histological sections and records from cohort members who developed gastric cancer subsequent to their multiphasic examination (cases) were reviewed and tumours were classified by histological type and site within the stomach as previously described.¹ Each confirmed case was then matched by five year birth cohort, sex, race, site, and date of serum collection (same month and year) to one cohort member (control) who had not developed cancer in the time up to and including the year of diagnosis. Serum samples were tested for *H pylori* antibodies as previously reported (Table I).^{1 23} Subjects with antibody titres higher than the mean of 15 low positive standards were considered to be infected with *H pylori*. From 107 cases of intestinal or diffuse type cancer of the corpus or antrum, 103 serum samples remained available for CagA antibody testing; 90 of these samples had been positive for *H pylori* antibodies by enzyme linked immunosorbent assay (ELISA). Controls included subjects previously matched to adenocarcinomas of the stomach and gastro-oesophageal junction and to gastric lymphomas. Among 147 control serum samples, eight were no longer available for testing. Of the remaining 139, 89 were from *H pylori* antibody positive subjects.

Serum samples from all subjects (*H pylori* positive and negative) were tested by ELISA

for antibodies to the *H pylori* CagA protein (OraVax, Inc, Cambridge, MS, USA) as previously described.^{17 22} This assay, which uses as antigen the orv220 fragment of CagA, is highly sensitive and specific for infection with CagA positive strains. Briefly, microtitre wells were coated with 1 µg orv220 antigen in carbonate-bicarbonate buffer. Test serum samples at a dilution of 1:100 were incubated in the wells at 37°C for one hour. The second antibody, a goat antihuman IgG alkaline phosphatase conjugate, was then added at a dilution of 1:1000 at 37°C for one hour. After incubation with phosphatase substrate, the optical density was read at 405 nm. All samples were run in triplicate. A titre was considered positive if the optical density exceeded the mean plus 3 SD of serum samples from 12 subjects without CagA positive infections. Using this cutoff point, we evaluated serum samples from 12 persons infected with CagA gene positive *H pylori*, nine persons infected with CagA gene negative *H pylori*, and five persons without *H pylori* by histology, culture, or rapid urease test. In this group, the assay was 100% sensitive and specific for CagA infection status.

In a previous study, serum samples had been tested for pepsinogen I²³; a concentration <50 ng/ml greatly increased the risk for cancer. This level is now categorised as "low pepsinogen I". Work by other investigators suggests that this low concentration of pepsinogen I detects moderate to severe corpus atrophy with 72% sensitivity and 85% specificity.^{24 25}

We evaluated level of education using three categories: less than high school, high school or trade school, and college or higher. We compared A v non-A ABO blood group and O v non-O ABO blood groups. Cigarette smoking had been determined by questionnaire when serum was collected. Information was categorised by current smoking status (current smoker, ex-smoker, non-smoker). Among smokers and ex-smokers, cigarette smoking was further categorised by duration of use (<10 years, 10-20 years, and >20 years) and quantity of cigarettes smoked daily (<one pack, one to two packs, and >two packs).

Data were analysed with EpiInfo (Centers for Disease Control, Atlanta, GA, USA) and Egret (Statistics and Epidemiology Research Corporation, Seattle, WA, USA) programs. For univariate comparisons between cases and controls, we used a χ^2 test with Yates's correction, *t* test, or Wilcoxon rank sum test. To evaluate whether infection with the specific phenotypes of *H pylori* increased risk for cancer compared with uninfected subjects, we performed logistic regression using the entire population of infected and uninfected subjects. In multivariate analysis, we adjusted for all variables previously used for matching including age at serum donation, sex, race, and date of serum donation. Subjects infected with each type of *H pylori* were omitted from the analysis of infection with the other type. Again, data were adjusted for age, sex, race, and date of serum collection. Interaction was investigated between all variables significant at $p<0.1$.

TABLE I Helicobacter pylori and CagA serology results for cancer cases and controls

	n	H pylori seropositive		H pylori seronegative	
		n (%)	n CagA+ (%)	n (%)	n CagA+ (%)*
Cases	103	90 (87.4)	70 (77.8)	13 (12.6)	1 (7.7)
Intestinal	77	67 (87.0)	54 (80.6)	10 (13.0)	1 (10)
Diffuse	26	23 (88.5)	16 (69.6)	3 (11.5)	0 (0)
Controls	139	89 (64.0)	48 (53.9)	50 (36.0)	4 (8.0)

*People with negative H pylori serology and positive CagA serology were excluded from analysis.

Gut: first published as 10.1136/gut.40.3.297 on 1 March 1997. Downloaded from <http://gut.bmj.com/> on April 25, 2023 by guest. Protected by copyright.

Results

A total of 103 cases (77 intestinal type and 26 diffuse type) and 139 controls were tested for CagA antibodies. At the time that serum was collected, those who later developed diffuse type cancer were younger than both controls and those who developed intestinal type cancer (Table II). Other demographic features were similar among cases and controls. Serum samples had been obtained a mean of 14.2 years before diagnosis of cancer in the cases.

Among subjects without *H pylori* antibodies, five (one case and four controls) had positive titres for CagA. Because of the ambiguity of these results, these subjects were excluded from further analysis. Among the remainder, *H pylori* infected subjects with CagA antibodies had a strongly increased risk for gastric cancer compared with uninfected subjects (Table III). This was true for both intestinal and diffuse type cancers. Risk for cancer was modestly but not significantly increased among CagA negative subjects infected with *H pylori* compared with uninfected subjects (Table III). When stratified by tumour type, however, CagA negative *H pylori* seemed to be statistically linked to diffuse type disease. The few subjects in this subgroup, however, make the risk estimate imprecise.

In a separate analysis we evaluated risk factors for cancer among only subjects infected with *H pylori*. Of the cases with *H pylori* antibodies, 77.8% also had antibodies to CagA compared with 53.9% of infected controls (odds ratio (OR) 3.3, 95% confidence interval (95% CI)=1.6–6.5). Seropositivity for CagA was significantly associated with gastric cancer (OR 3.3, 95% CI=1.6–6.5). Among cases of intestinal type cancer, the OR was 3.8 (95% CI=1.7–8.4) whereas among cases of diffuse cancer the OR was lower and not significant (OR 2.1, 95% CI=0.7–6.1).

Of the 179 subjects infected with *H pylori*, 159 had had serum tested for pepsinogens; of these 50 (31.3%) had pepsinogen I concentrations <50 ng/ml. Thirty nine (36.4%) of 107 subjects with CagA antibodies had low pepsinogen I concentrations at the time the serum was obtained compared with 11 of 52 (22.4%) subjects without CagA antibodies (p=0.1). When pepsinogen I was added to the multivariate model of CagA, CagA seropositivity remained a significant risk factor for intestinal type cancer, although the magnitude of the association decreased (Table IV). Low pepsinogen I was strongly associated with later development of both intestinal and diffuse type malignancy.

If CagA protein caused cancer uniquely through induction of atrophy, then the association between CagA and cancer should tend to weaken as the interval between serum collection and cancer diagnosis lessened; conversely, the association between low pepsinogen and cancer would be expected to increase. The opposite was observed. In 41 cases with 15 years or more between serum collection and cancer diagnosis, CagA only increased risk for cancer 1.2-fold after adjustment for pepsinogen concentration (95% CI=0.5–3.0) whereas low pepsinogen I was a strong risk factor for cancer (OR 6.3, 95% CI=2.4–16.7). In 41 cases with an interval <15 years between serum collection and cancer the OR was 3.8 for CagA (95% CI=1.3–10.5) and 4.1 for low pepsinogen I (95% CI=1.5–10.7).

In no analysis among *H pylori* infected cases and controls was the level of educational attainment, cigarette smoking, or ABO blood group significantly associated with development of malignancy (Table V). Because type A blood seemed to be somewhat more common in cases than in controls and because this blood group has been identified as a risk factor only for diffuse cancer, we stratified our analysis by tumour type. The association between type A

TABLE II Demographic features of cases and controls

Feature	Cancer		Controls (n=139)
	Intestinal (n=77)	Diffuse (n=26)	
Female (%)	29.9	39.1	24.7
Race (%):			
White	65.7	73.9	73.0
African-American	32.8	17.4	27.7
Asian	1.5	8.7	2.2
Level of education (%):*			
Elementary and grade school	35.5	22.7	32.6
High school or trade school	37.1	45.5	37.1
College education	27.4	26.7	25.8
ABO blood group (%):†			
O	43.9	43.5	50.0
A	40.9	43.5	31.8
B	12.1	13.0	12.5
AB	3.0	0.0	5.6
Cigarette smoking at time of serum collection (%):‡			
Smoker	33.9	37.5	29.3
Ex-smoker	18.6	12.5	29.3
Non-smoker	47.4	50.0	41.3
Mean age when serum was obtained	56.0§	50.7	56.1§
Median <i>H pylori</i> IgG titre¶	3.1	3.1	3.5
Low pepsinogen (%)	45.0	40.9	15.6§**
Mean age diagnosis of cancer	70.1	67.4	—

*Data missing for five intestinal cases, one diffuse case and five controls.
†Data missing for one intestinal case and one control.
‡Data missing for eight intestinal cases, seven diffuse cases, and 14 controls.
§p<0.05 v diffuse cancer cases.
¶Value is expressed as the ratio of the sample titre to the mean of three low positive standards.
|| Defined as a serum pepsinogen I concentration <50 ng/ml. Information missing for seven intestinal cases, one diffuse case, and 12 controls.
**p<0.05 v intestinal cancer cases.

TABLE III Risk for stomach cancer in people with CagA positive H pylori infection and CagA negative H pylori infection compared with uninfected controls*

	CagA positive v uninfected			CagA negative v uninfected		
	Cases with H pylori n (%)	Controls with H pylori n (%)	OR* (95% CI)	Cases with H pylori n (%)	Controls with H pylori n (%)	OR* (95% CI)
All cancers	70/82 (85.4)	48/94 (51.1)	5.8 (2.6 to 13.0)	20/32 (62.5)	41/87 (47.1)	2.2 (0.9 to 5.4)
Intestinal cancer	54/63 (85.7)	48/94 (51.1)	5.1 (2.1 to 12.1)	13/22 (59.1)	41/87 (47.1)	1.4 (0.5 to 3.9)
Diffuse cancer	16/19 (84.2)	48/94 (51.1)	10.1 (2.7 to 47.5)	7/10 (70.0)	41/87 (47.1)	9.0 (1.2 to 65.8)

*Analysis adjusted for age, sex, race, and date of serum collection with the exception of the analysis of diffuse cancer in CagA negative v uninfected groups. Because of small numbers, this analysis could not be adjusted by race.

TABLE IV Risk for gastric cancer in subjects infected with *H pylori* associated with CagA antibody and low pepsinogen I concentration (<50 ng/ml), unadjusted for each other and adjusted for each other*

	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	p Value
Intestinal cancer:			
CagA antibodies	3.1 (1.4 to 7.1)	2.6† (1.1 to 6.1)	0.03
Low pepsinogen I	5.2 (2.2 to 12.0)	4.7 (2.0 to 11.0)	<0.001
Diffuse cancer:			
CagA antibodies	1.7 (0.6 to 5.0)	1.3‡ (0.4 to 4.1)	0.6
Low pepsinogen I	7.0 (1.9 to 25.7)	6.7 (1.8 to 25.0)	0.005
All cancers:			
CagA antibodies	2.6 (1.3 to 5.5)	2.3 (1.0 to 5.9)§	0.04
Low pepsinogen I	5.5 (2.5 to 12.3)	5.1 (2.3 to 11.6)	<0.001

*Excludes two intestinal cases, one diffuse case, and 12 controls from whom pepsinogen I concentrations were not available. All analyses were adjusted for age, sex, race, and date of serum donation.
†OR for CagA without adjustment for pepsinogen=3.3 (95% CI 1.4 to 7.5).
‡OR for CagA without adjustment for pepsinogen=1.8 (95% CI 0.6 to 5.4).
§OR for CagA without adjustment for pepsinogen=2.8 (95% CI 1.3 to 5.8).

TABLE V Risk for gastric cancer associated with education, ABO blood group, and cigarette smoking among people infected with *H pylori*

	H pylori+/CagA+ v H pylori+/CagA- OR (95% CI)	H pylori+/CagA+ v uninfected OR (95% CI)	H pylori+/CagA- v uninfected OR (95% CI)
Level of education:			
Elementary and grade school	1.0	1.0	1.0
High school or trade school	0.9 (0.4 to 2.0)	0.7 (0.3 to 1.5)	1.5 (0.5 to 4.5)
College education	0.9 (0.4 to 2.2)	0.6 (0.3 to 1.5)	1.2 (0.4 to 3.9)
Cigarette use:			
Non-smoker	1.0	1.0	1.0
Ex-smoker	0.5 (0.2 to 1.2)	0.7 (0.3 to 1.7)	0.6 (0.2 to 2.1)
Current smoker	1.1 (0.5 to 2.6)	0.9 (0.4 to 2.2)	1.1 (0.4 to 3.2)
ABO Blood group:			
Blood group not A	1.0	1.0	1.0
Blood group A	1.7 (0.9 to 3.3)	1.3 (0.6 to 2.5)	1.1 (0.4 to 2.5)

*All analyses adjusted for CagA antibody, age, sex, race, and date of serum donation.

blood and cancer was similar in both tumour subgroups (OR for intestinal cancer 1.9, 95% CI=0.9–3.9; OR for diffuse cancer 1.6, 95% CI 0.6–4.4).

Discussion

Helicobacter pylori infection causes both intestinal and diffuse types of gastric adenocarcinoma. The organism's carcinogenic effects have been imputed to induction of inflammation. Yet not all *H pylori* strains cause similar inflammatory changes. The phenotype of *H pylori* that expresses CagA causes higher degrees of acute and chronic inflammation than the CagA negative phenotype, for unknown reasons.^{16 18 26–28} Our study suggests that this difference in phenotype impacts the risk for malignancy. Overall, CagA positive strains were three times more likely to lead to cancer than CagA negative strains. This only held true in the subgroup of intestinal type cancers. Moreover, when compared with uninfected subjects, persons infected with CagA negative strains had only a modest but not significant, increase in their risk for cancer. When evaluated by tumour type, however, an interesting pattern was seen. Both phenotypes of *H pylori* seemed to increase risk for diffuse type cancer but only the CagA phenotype was associated with intestinal type malignancy. This suggests that the mechanisms of carcinogenesis imputed to *H pylori* differ for the two types of cancer. Some likely confounders measured in this study (socioeconomic status

(as indicated by level of education), cigarette smoking, and ABO blood group) did not explain these associations.

Among the reasons *H pylori* strains may show variation in leading to cancer is a differing predilection to cause atrophic gastritis. Atrophy has been shown to occur more commonly when the infecting strain expresses cytotoxin than when it does not.²⁹ Although cytotoxin and CagA are not perfectly associated, cytotoxin expression is statistically much more common when the CagA gene is also present and expressed.^{30 31} Moreover, a recent prospective study showed more frequent progression to atrophic gastritis in subjects with CagA positive *H pylori* infection than in CagA negative infected hosts.²¹ CagA positive strains may thus set in action the chain of events that results in intestinal type malignancy. Whereas pepsinogen I concentration is an imperfect marker of atrophy, concentrations below 50 ng/ml have been reported to indicate moderate to severe corpus atrophy.²⁵ In this study, nearly one fourth of the excess risk associated with CagA seropositivity could be explained by concomitant low pepsinogen concentrations. Misclassification of atrophy by use of the pepsinogen assay rather than direct examination of the mucosa would tend to underestimate this effect. The use of serum samples obtained almost 15 years before diagnosis of cancer could also potentially underestimate the true relation between CagA and atrophic gastritis. Development of corpus atrophy during the interval years would have been missed. Yet when we stratified our analysis by interval from serum collection to diagnosis of cancer, there were no trends in the associations among CagA, atrophy, and cancer. Thus whereas a proportion of cancer risk associated with CagA positive *H pylori* seems to be due to its link with atrophic gastritis, our data suggest other carcinogenic effects of CagA positive strains independent of this pathway.

Diffuse type cancers have not been traditionally thought to arise after corpus atrophy but to arise de novo from non-atrophic tissue.⁴ We had little tissue with which to histopathologically differentiate between intestinal and diffuse cancers. The presence of glandular forms in tumours, therefore, could have been missed and diffuse type cancer overdiagnosed. Such misclassification would tend to bias the results towards finding associations between atrophy and diffuse malignancy where none truly existed. On the other hand, our cases of diffuse cancer were similar demographically to diffuse cancers described in other studies. The patients were younger than those with intestinal cancer, more equally distributed between males and females, and had a higher prevalence of type A blood.¹ We doubt that misclassification can account entirely for the findings in this study, but the exact association between CagA, pepsinogen, and diffuse malignancy remains open to question.

In the past 10 years, investigators have begun to piece together the many factors that contribute to gastric carcinogenesis. Among those infected with *H pylori*, two factors

Gut: first published as 10.1136/gut.40.3.297 on 1 March 1997. Downloaded from <http://gut.bmj.com/> on April 25, 2023 by guest. Protected by copyright.

enhance risk: seropositivity to CagA and low pepsinogen concentration. Preliminary data indicate that the CagA phenotype is not equally common in all populations.^{32 33} Better understanding of the distribution and interactions among these factors may foster development of intervention strategies to prevent this often fatal malignancy.

We thank OraVax for generously donating the orv220 CagA antigen used in this study. We also thank Ms Shufang Yang and Ms Marilyn Replogle for their technical assistance.

This study was supported by a clinical epidemiology fellowship from Merck and the Society for Epidemiologic Research (JP) and by NIH grant R35CA49761 from the National Cancer Institute (GDF).

- 1 Parsonnet J, Friedman GD, Vandersteen DP, *et al.* Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; 325: 1127–31.
- 2 Nomura AMY, Stemmerman GN, Chyou P, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma in a population of Japanese-Americans in Hawaii. *N Engl J Med* 1991; 325: 1132–6.
- 3 IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Helicobacter pylori. In: *Schistosomes, liver flukes, and Helicobacter pylori: views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans*. Lyon: IARC, 1994: 177–240.
- 4 Nomura AM, Stemmermann GN, Chyou PH. Gastric cancer among the Japanese in Hawaii. *Jpn J Cancer Res* 1995; 86: 916–23.
- 5 Kneller RW, You WC, Chang YS, *et al.* Cigarette smoking and other risk factors for progression of precancerous stomach lesions. *J Natl Cancer Inst* 1992; 84: 1261–6.
- 6 Hansson LE, Baron J, Nyren O, Bergstrom R, Wolk A, Adami HO. Tobacco, alcohol, and the risk of gastric cancer. A population-based case-control study in Sweden. *Int J Cancer* 1994; 57: 26–31.
- 7 Howson C, Hiyama T, Wynder E. The decline in gastric cancer: epidemiology of an unplanned triumph. *Epidemiol Rev* 1986; 8: 1–27.
- 8 Boren T, Falk P, Roth KA, Larson G, Normack S. Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. *Science* 1993; 262: 1892–5.
- 9 Mentis A, Blackwell CC, Weir DM, Spiliadis C, Dailianas A, Skandalis N. ABO blood group, secretor status and detection of Helicobacter pylori among patients with gastric or duodenal ulcers. *Epidemiol Infect* 1991; 106: 221–9.
- 10 Cover TL, Tummuru MK, Cao P, Thompson SA, Blaser MJ. Divergence of genetic sequences for the vacuolating cytotoxin among Helicobacter pylori strains. *J Biol Chem* 1994; 269: 10566–73.
- 11 Takami S, Hayashi T, Tonokatsu Y, Shimoyama T, Tamura T. Chromosomal heterogeneity of Helicobacter pylori isolates by pulsed-field gel electrophoresis. *Int J Med Microbiol Virol Parasitol Infect Dis* 1993; 280: 102–7.
- 12 Desai M, Linton D, Owen RJ, Cameron H, Stanley J. Genetic diversity of Helicobacter pylori indexed with respect to clinical symptomatology, using a 16S rRNA and a species-specific DNA probe. *J Appl Bacteriol* 1993; 75: 574–82.
- 13 Taylor DE, Eaton M, Chang N, Salama SM. Construction of a Helicobacter pylori genome map and demonstration of diversity at the genome level. *J Bacteriol* 1992; 174: 6800–6.
- 14 Telford JL, Covacci A, Ghiara P, Montecucco C, Rappuoli R. Unravelling the pathogenic role of Helicobacter pylori in peptic ulcer: potential new therapies and vaccines. *Trends Biotechnol* 1994; 12: 420–6.
- 15 Xiang Z, Censini S, Bayeli PF, *et al.* Analysis of expression of CagA and VacA virulence factors in 43 strains of Helicobacter pylori reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin. *Infect Immun* 1995; 63: 94–8.
- 16 Crabtree JE, Covacci A, Farmery SM, *et al.* Helicobacter pylori induced interleukin-8 expression in gastric epithelial cells is associated with CagA positive phenotype. *J Clin Pathol* 1995; 48: 41–5.
- 17 Cover TL, Glupczynski Y, Lage AP, *et al.* Serologic detection of infection with cagA+ Helicobacter pylori strains. *J Clin Microbiol* 1995; 33: 1496–500.
- 18 Crabtree JE, Taylor JD, Wyatt JJ, *et al.* Mucosal IgA recognition of Helicobacter pylori 120 kDa protein, peptic ulceration, and gastric pathology. *Lancet* 1991; 338: 332–5.
- 19 Peek RM, Miller GG, Tham KT, *et al.* Heightened inflammatory response and cytokine expression in vivo to cagA+ Helicobacter pylori strains. *Lab Invest* 1995; 73: 760–70.
- 20 Blaser MJ. Intrastrain differences in Helicobacter pylori: a key question in mucosal damage? *Ann Med* 1995; 27: 559–63.
- 21 Kuipers EJ, Perez-Perez GI, Meuwissen SG, Blaser MJ. Helicobacter pylori and atrophic gastritis: importance of the cagA status. *J Natl Cancer Inst* 1995; 87: 1777–80.
- 22 Blaser MJ, Perez-Perez GI, Kleanthous H, *et al.* Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; 55: 2111–5.
- 23 Parsonnet J, Samloff IM, Nelson LM, Orentreich N, Vogelstein JH, Friedman GD. Helicobacter pylori, pepsinogen and risk for gastric adenocarcinoma. *Cancer Epidemiol Biomarkers Prev* 1993; 2: 1–7.
- 24 Borch K, Axelsson K, Halgreen H, Damkjaer Nielsen MD, Ledin T, Szesci PB. The ratio of pepsinogen A to pepsinogen C: a sensitive test for atrophic gastritis. *Scand J Gastroenterol* 1989; 24: 870–6.
- 25 Kekki M, Samloff IM, Varis K, Ihamaki T. Serum pepsinogen I and serum gastrin in the screening of severe atrophic gastritis. *Scand J Gastroenterol Suppl* 1991; 186: 109–16.
- 26 Huang J, O'Toole PW, Doig P, Trust TJ. Stimulation of interleukin-8 production in epithelial cell lines by Helicobacter pylori. *Infect Immun* 1995; 63: 1732–8.
- 27 Sharma SA, Tummuru MK, Miller GG, Blaser MJ. Interleukin-8 response of gastric epithelial cell lines to Helicobacter pylori stimulation in vitro. *Infect Immun* 1995; 63: 1681–7.
- 28 Blaser MJ. Helicobacter pylori phenotypes associated with peptic ulceration. *Scand J Gastroenterol Suppl* 1994; 205: 1–5.
- 29 Fox JG, Correa P, Taylor NS, *et al.* High prevalence and persistence of cytotoxin-positive Helicobacter pylori strains in a population with high prevalence of atrophic gastritis. *Am J Gastroenterol* 1992; 87: 1554–60.
- 30 Atherton JC, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; 270: 17771–7.
- 31 Xiang Z, Censini S, Bayeli PF, *et al.* Analysis of expression of CagA and VacA virulence factors in 43 strains of Helicobacter pylori reveals that clinical isolates can be divided into two major types and that CagA is not necessary for expression of the vacuolating cytotoxin. *Infect Immun* 1995; 63: 94–8.
- 32 Fauconnier A, Burette A, Goutier S, Butzler JP, Bollen A, Glupczynski Y. Frequency of cagA in Helicobacter pylori strains isolated from Belgian and Moroccan patients [abstract]. *Gut* 1995; 37: A69.
- 33 Parsonnet J, Replogle ML, Yang S, Hiatt RA. Prevalence of the type I H. pylori phenotype differs among ethnic/racial groups in the San Francisco Bay area. *J Infect Dis* (in press).