Ischaemic heart disease and Cag A strains of *Helicobacter pylori* in the Caerphilly heart disease study

A F M Stone, P Risley, H S Markus, B K Butland, D P Strachan, P C Elwood, M A Mendall

Abstract

Objective—To look for the presence of the more virulent strains of *Helicobacter pylori* (*H pylori*) in men who developed ischaemic heart disease over a 10 year period and in controls.

Design—The Caerphilly prospective heart disease study recruited 2512 men aged 45–59 years during 1979–83. Western blot analysis or enzyme linked immunosorbent assay (ELISA) was performed on serum taken from those who subsequently died of ischaemic heart disease, or developed non-fatal myocardial infarction, to determine *H pylori* and Cag A status. Similar information was available on age matched controls.

Results—During the first decade of the study, 312 men died of ischaemic heart disease or developed non-fatal myocardial infarction. Serum was available from 172 of these (55%). There was no evidence of an association between Cag A seropositivity and incident ischaemic heart disease or ischaemic heart disease mortality, either before or after adjustment for potential confounders (adjusted odds ratios 1.18 (95% confidence interval (CI) 0.76 to 1.85) and 1.13 (95% CI 0.61 to 2.07), respectively). Further, the odds ratios for ischaemic heart disease incidence and ischaemic heart disease mortality by *H pylori* seropositivity did not appear to depend on the presence or absence of Cag A strains (p = 0.76 and 0.77, respectively).

Conclusions—In this cohort of middle aged men, followed over a 10 year period, there is little evidence of an association between Cag A seropositivity and either incident ischaemic heart disease or ischaemic heart disease mortality.

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Division of Gastroenterology, Endocrinology and Metabolism, St George's Hospital Medical School, London SW17, UK
A F M Stone
P Risley

Department of Clinical Neurosciences, St George's Hospital Medical School
H S Markus

Department of Public Health Sciences, St George's Hospital Medical School
B K Butland
D P Strachan

Medical Research Council Epidemiology Unit (South Wales), Llandough Hospital, South Glamorgan, UK
P C Elwood

Mayday University Hospital, Mayday Rd, Thornton Heath, Surrey CR7 7YE, UK
M A Mendall

Correspondence to:
Dr Mendall
mmendall@sghms.ac.uk

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Helicobacter pylori is a lifelong bacterial infection of the stomach that is mainly acquired in childhood.[1] Over 20 epidemiological studies have been reported that have investigated the role of *H pylori* in the aetiology of ischaemic heart disease.[2] Early case–control studies[3] broadly supported an association. Cross sectional population studies have given more mixed findings, while more detailed information has recently come from large population based prospective case–control studies. These have attempted to overcome the bias present in case–control and cross sectional studies, and are consistent with only very modest effects. A meta-analysis performed on the seven previous prospective studies involving *H pylori* and ischaemic heart disease covered 2286 cases. A combined analysis showed an adjusted odds ratio of 1.16 (95% confidence interval (CI) 0.94 to 1.43).[4]

The inconsistent results may stem from the fact that bacterial and host factors influence the risk, and these have not been assessed in the studies to date. There is a great variability in the severity of *H pylori* associated gastritis, both between individuals and also at varying stages of life within the individual. Gastritis is more severe in young and early middle aged people, and *H pylori* may be a stronger risk factor for ischaemic heart disease in younger subjects.[5]

The *H pylori* cytotoxin associated gene product A (Cag A) is a marker for enhanced virulence and increased cytotoxin production, and is more commonly found in strains of *H pylori* causing more severe gastritis, peptic ulcer disease, and gastric cancer.[6] There are conflicting results from studies designed to investigate the link between CagA seropositivity and ischaemic heart disease, but the results are consistent with a weak association. If confirmed, the finding of a "dose–response" relation between *H pylori* and ischaemic heart disease would greatly strengthen the likelihood of a causal association, allaying fears of residual confounding by social class.

We measured serum antibodies to Cag A protein using serum collected during a prospective study of middle aged men in whom a wide variety of conventional cardiovascular risk factors were known. We related these results to fatal or non-fatal ischaemic heart disease over a 10 year period.

Methods

The Caerphilly prospective heart disease study[7] targeted all men aged 45–59 years in the town of Caerphilly and five adjacent villages in south Wales during 1979–83, and recruited 89% (2512) of the 2818 eligible men contacted. Symptoms and ECG abnormalities suggestive of past or current ischaemic heart disease were ascertained and a range of cardiovascular risk factors was measured, including smoking history, standing height, body weight, blood pressure, forced expiratory volume in

one second (FEV_1), total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, fibrinogen, plasma viscosity, and leucocyte count. Socioeconomic status was derived from each man's current occupation and his father's occupation during childhood and classified according to the registrar general's social classes. Subjects were followed up at five year intervals.

Fasting venous blood was collected at entry into the study and serum stored at −20°C, with one thaw cycle in the intervening years. Specimens were unavailable for approximately one half of the cohort owing to depletion of material caused by previous studies. *H pylori* IgG data were taken from our previous study of the whole cohort[8] which used a commercially available enzyme linked immunosorbent assay (ELISA; Helicobacter pylori HM-CAP, Sigma Diagnostics, St Louis, Missouri, USA) to measure anti *H pylori* immunoglobulin G. Cag A status was determined using western blot analysis (HelicoBlot 2.0, Genelabs Diagnostics, Singapore). Where availability was very limited (23% of cases, 18% of controls) samples were assayed by ELISA (Helicobacter p120 Cag A ELISA, Viva Diagnostika, Germany). Both the Cag A assays had been previously validated in our laboratory by comparison with the presence of *H pylori* on histology of gastric antral biopsies, and CLO testing, in 48 patients undergoing upper gastrointestinal endoscopy. Patients were assumed to be infected with *H pylori* if both histology and CLO test were positive, and to be uninfected if both were negative. This was to ensure local validation of the antigens used. The strain of *H pylori* was assumed to be positive for Cag A if infection was associated with duodenal ulceration[9]. Of 17 *H pylori* positive subjects with duodenal ulceration, all were positive for Cag A with the HelicoBlot assay and 16 of 17 were positive on Cag A ELISA. Of the 24 *H pylori* negative subjects, 21 were negative on both HelicoBlot and Cag A ELISA. The remaining seven subjects were discounted owing to discrepancy between the histological findings and CLO test result.

Deaths were classified according to the ninth revision of the *International Classification of Diseases* (ICD9) as caused by ischaemic heart disease (ICD9 410-414). Incident myocardial infarction was ascertained from review of hospital notes and ECG changes. Three groups were therefore included:

- fatal ischaemic heart disease
- clinical myocardial infarction (admitted to hospital with episodes meeting World Health Organization criteria of combinations of serial ECG changes, cardiac enzyme abnormalities, and acute symptoms)
- ECG evidence of myocardial infarction with development of new Q or QS waves on follow up ECG in the absence of Q or QS waves on the ECG recorded at entry.

STATISTICAL METHODS

The data were analysed as for an unmatched case–control study with *H pylori* strain defined as the three categories Hp−, Hp+/Cag A−, and Hp+/Cag A+. Cross tabulations of seropositivity by incident ischaemic heart disease were produced in STATA.[10]

Table 1 Seropositivity among cases and controls

		Incident IHD	
H pylori status	*Cag A*	*Cases (IHD deaths)*	*Controls*
Positive	Positive	87 (41)	95
	Negative	42 (26)	48
Negative	Positive	3 (0)	4
	Negative	40 (13)	58
Total	Positive	90 (41)	99
	Negative	82 (39)	106

IHD, ischaemic heart disease.

Logistic regression in STATA was used to adjust associations between seropositivity and incident ischaemic heart disease, or ischaemic heart disease mortality, for the confounding effects of other potential coronary risk factors. These included age, body mass index, smoking history, systolic blood pressure, total cholesterol, social class currently and in childhood, standing height, and mean FEV_1. The consequences of additional adjustment for possible intermediates (for example, fibrinogen, white cell count) were also investigated.

H pylori was modelled both as a two level factor (Hp−, Hp+) and as a three level factor (Hp−, Hp+/Cag A−, Hp+/Cag A+). Differences in fit between these two and three factor logistic regression models were used to test for a difference in the odds of disease associated with Cag A compared with other *H pylori* strains.

Results

During the first decade of the study, 312 men died of ischaemic heart disease or developed non-fatal myocardial infarction. Of these, 172 (55%) had complete *H pylori* and Cag A status data. Similar data were available on 205 age matched controls, with no incident ischaemic heart disease by the time of the case event.

There were 233 men who were seropositive for *H pylori* and 105 who were seronegative. Seven men were *H pylori* negative and Cag A positive and were therefore excluded. Table 1 shows the serological status for *H pylori* and Cag A among cases and controls. There was no evidence of an association between Cag A seropositivity and death from ischaemic heart disease (unadjusted odds ratio 1.17, 95% CI 0.70 to 1.97, p = 0.55; adjusted odds ratio 1.13, 95% CI 0.61 to 2.07, p = 0.70). Similarly, table 2 shows no evidence of a positive association between Cag A seropositivity and incident ischaemic heart disease, either before or after adjustment for potential confounders including age, smoking habit, body mass index, systolic blood pressure, total cholesterol, social class, height, and lung function (adjusted odds ratio 1.18, 95% CI 0.76 to 1.85).

Table 3 shows logistic regression analysis for incident ischaemic heart disease by *H pylori* strain, adjusting for potential cardiovascular risk factors. The odds ratio for incident ischaemic heart disease by *H pylori* seropositivity did

Table 2 Logistic regression analysis for incident ischaemic heart disease by Cag A seropositivity

Adjustment	*OR*	*95% CI*	*p Value*
All available data (n=370)			
(1) Unadjusted	1.17	(0.77 to 1.78)	0.47
Data restricted to subjects with complete information on confounders (n=354)			
(2) Model 1 plus age, BMI, systolic blood pressure, smoking history and total cholesterol	1.17	(0.76 to 1.82)	0.48
(3) Model 2 plus own and father's social class	1.22	(0.78 to 1.91)	0.38
(4) Model 3 plus height and lung function	1.18	(0.76 to 1.85)	0.46
(5) Model 4 plus fibrinogen and white cell count	1.23	(0.78 to 1.95)	0.37

OR, odds ratio; CI, confidence interval; BMI, body mass index.

not appear to depend upon *H pylori* strain, either before or after adjustment for confounders (p = 0.86 and p = 0.76, respectively). In other words, among men seropositive for *H pylori*, there was no evidence of an association between ischaemic heart disease and Cag A. For ischaemic heart disease mortality, the unadjusted odds ratio for Hp+/CagA− *v* Hp− was slightly higher than for Hp+/Cag A+ *v* Hp− (odds ratios 2.42, 95% CI 1.12 to 5.21, and 1.93, 95% CI 0.95 to 3.89, respectively). This difference was in the opposite direction to that hypothesised and was not significant (p = 0.46). After adjustment for potential confounders the odds ratios were 1.73 (95% CI 0.70 to 4.26) and 1.56 (95% CI 0.68 to 3.54), respectively (test for difference in odds ratios: p = 0.77).

Discussion

This nested case–control study has shown no evidence of a strong association between Cag A seropositivity and ischaemic heart disease (incident or fatal). Unfortunately, serum was available from only 172 of the 312 men (55%) who developed ischaemic heart disease during the study period. However, this group was quite similar to the remaining 45%, sharing the same mean age (53 years) and containing only a slightly higher proportion of men from non-manual social classes (30% *v* 24%). Our results failed to support the findings of Pasceri and colleagues,[11] who studied 88 patients with angiographically confirmed ischaemic heart disease and compared them with blood donor controls. They reported an adjusted odds ratio of 3.8 (95% CI 1.6 to 9.1) for ischaemic heart disease by Cag A seropositivity. They also showed no relation between infection with Cag A negative strains and ischaemic heart disease.

Our findings do, however, agree with four more recent studies.

The British regional heart study[4] compared 505 male ischaemic heart disease cases with 1025 controls. Subjects, aged 40–59 years, were recruited from general practitioner lists in 24 British towns, and 90% of survivors were traced at 10 years. Their results suggested no strong relation between the incidence of ischaemic heart disease and Cag A positive *H pylori* infection, giving an odds ratio of 1.10 (95% CI 0.71 to 1.71). A similar odds ratio was found when comparisons of Cag A seropositivity was restricted to only the *H pylori* positive individuals.

A further study[12] looked at 312 subjects aged 40–68 years with evidence of ischaemic heart disease on elective angiography. These were compared with 479 blood donor controls. The adjusted odds ratio for ischaemic heart disease among Cag A seropositive patients, relative to *H pylori* seronegative controls, was 1.1 (95% CI 0.7 to 1.7).

A third study[13] looked at 342 cases of acute myocardial infarction and 214 population based controls. No significant association was found between Cag A seropositivity and the incidence of acute myocardial infarction, although subgroup analysis found a significant association in subjects under the age of 55, with an odds ratio of 2.25 (95% CI 1.12 to 4.53) (p = 0.01).

The most recently published study[14] compared 259 cases of myocardial infarction with the same number of population based controls and, after adjustments, reported an odds ratio of 1.16 (95% CI 0.79 to 1.70).

Previous analysis of the Caerphilly study[8] suggested that *H pylori* was unlikely to be a strong risk factor for ischaemic heart disease but raised the possibility of an increased risk of death, from both ischaemic heart disease and other causes, among men seropositive for *H pylori*. Our study failed to support the hypothesis that the virulence of the strain of *H pylori* had significant effect upon that increased risk of death.

If the studies to date are consistent with a weak association between *H pylori* infection and ischaemic heart disease, it does not appear that the virulence of the strain of *H pylori* is an important factor. Apart from the interpretation that there is no association between *H pylori*

Table 3 Logistic regression analysis for incident ischaemic heart disease by H pylori strain

Adjustment	*Hp+ and CagA− v Hp−*		*Hp+ and CagA+ v Hp−*		*Test* for difference between odds ratios (Ho:OR_1 = OR_2)*
	OR_1	*95% CI*	*OR_2*	*95% CI*	
All available data (n=370) (1) Unadjusted	1.27	(0.71 to 2.26)	1.33	(0.81 to 2.18)	0.86
Data restricted to subjects with complete information on confounders (n=354)					
(1) Unadjusted	1.26	(0.70 to 2.28)	1.31	(0.78 to 2.17)	0.89
(2) Model 1 plus age, BMI, systolic blood pressure, smoking history, and total cholesterol	1.07	(0.57 to 2.03)	1.21	(0.71 to 2.07)	0.66
(3) Model 2 plus own and father's social class	1.19	(0.61 to 2.29)	1.33	(0.77 to 2.30)	0.69
(4) Model 3 plus height and lung function	1.17	(0.61 to 2.28)	1.28	(0.74 to 2.23)	0.76
(5) Model 4 plus fibrinogen and white cell count	1.25	(0.64 to 2.46)	1.38	(0.78 to 2.43)	0.75

*Based on difference in fit between logistic regression models, one containing a two level factor for *H pylori* (Hp+, Hp−) and one containing a three level factor for *H pylori* (Hp−, Hp+/Cag A−, Hp+/Cag A+).
BMI, body mass index; CI, confidence interval; OR, odds ratio.

and ischaemic heart disease, there are two other possible explanations for this finding.

H pylori could increase cardiovascular risk through mechanisms independent of its gastric inflammatory effects. Immunological cross reactivity has been suggested as one possible mechanism. Birnie and colleagues reported a correlation between antibodies to anti-heat shock protein 65 (hsp65), an endothelial antigen, and both the severity and extent of coronary atherosclerosis.[15] They showed that anti-hsp65 titres correlated well with anti-*H pylori* titres and successful eradication of *H pylori* led to a significant fall in anti-hsp65 titres. Analysis of our data showed that among *H pylori* positive subjects, hsp65 antibody levels were somewhat ($p = 0.06$) higher in those with Cag A seropositive strains (mean (SD), 0.86 (0.42) optical density (OD) units) than in those with Cag A negative strains (mean (SD), 0.74 (0.33) OD units). However, as previously reported,[8] there was no substantial or significant difference between all seropositive subjects and all those seronegative. Therefore, it is unlikely that an autoimmune response, with antibodies against a bacterial protein cross reacting with hsp65, would generate an association of ischaemic heart disease with *H pylori* infection but not with Cag A serotype.

Another possible explanation could be the age of the subject. Subjects infected with CagA bearing strains are more likely to develop atrophic gastritis and intestinal metaplasia by late middle age, which is possibly a less inflamed state. This may explain earlier findings of a stronger association of *H pylori* with ischaemic heart disease in younger subjects.[5] Indeed there were suggestions in the case–control study by Koenig and colleagues that, in subjects less that 60 years old, the effect may be stronger, but the numbers were too small to analyse this convincingly.[12]

CONCLUSIONS

It is unlikely that there is a strong association between *H pylori* and cardiovascular disease. Studies of Cag A serotypes do not remove fears over residual confounding by socioeconomic status.

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