CagA+ *Helicobacter pylori* infection and gastric cancer risk in the EPIC-EURGAST study

Domenico Palli[1]*, Giovanna Masala[1], Giuseppe Del Giudice[2], Mario Plebani[3], Daniela Basso[3], Duccio Berti[2], Mattijs E. Numans[4], Marco Ceroti[1], Petra H.M. Peeters[4], H. Bas Bueno de Mesquita[5], Frederike L. Buchner[5], Francoise Clavel-Chapelon[6], Marie-Christine Boutron-Ruault[6], Vittorio Krogh[7], Calogero Saieva[1], Paolo Vineis[8,9], Salvatore Panico[10], Rosario Tumino[11], Olof Nyrén[12], Henrik Simán[13], Goran Berglund[13], Goran Hallmans[14], Maria-Jose Sanchez[15], Nerea Larrãnaga[16], Aurelio Barricarte[17], Carmen Navarro[18], Jose R. Quiros[19], Tim Key[20], Naomi Allen[20], Sheila Bingham[21], Kay Tee Khaw[22], Heiner Boeing[23], Cornelia Weikert[23], Jakob Linseisen[24], Gabriele Nagel[24], Kim Overvad[25], Reimar W. Thomsen[25], Anne Tjonneland[26], Anja Olsen[26], Antonia Trichoupoulou[27], Dimitrios Trichopoulos[27], Athina Arvaniti[27], Guillem Pera[28], Rudolf Kaaks[29], Mazda Jenab[29], Pietro Ferrari[29], Gabriella Nesi[30], Fatima Carneiro[31], Elio Riboli[9,29] and Carlos A. Gonzalez[28]

[1]*Molecular and Nutritional Epidemiology Unit, CSPO, Scientific Institute of Tuscany, Florence, Italy*
[2]*Research Center, Novartis Vaccines, Siena, Italy*
[3]*Department of Laboratory Medicine, University Hospital, Padua, Italy*
[4]*Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, The Netherlands*
[5]*Center for Nutrition and Health, National Institute for Public Health and the Environment, Bilthoven, The Netherlands*
[6]*INSERM, Institut Gustave Roussy, Villejuif, France*
[7]*Epidemiology Unit, Istituto Nazionale Tumori, Milan, Italy*
[8]*CPO and University of Turin, Turin, Italy*
[9]*Department of Epidemiology and Public Health, Imperial College, London, United Kingdom*
[10]*Dipartimento di Medicina Clinica e Sperimentale, Federico II University, Naples, Italy*
[11]*Cancer Registry, Azienda Ospedaliera ''Civile M.P. Arezzo'', Ragusa, Italy*
[12]*Department of Medical Epidemiology, Karolinska Institutet, Stockholm, Sweden*
[13]*Department of Medicine, University Hospital Malmö, Lund University, Malmö, Sweden*
[14]*Department of Nutritional Research, University of Umeå, Umeå, Sweden*
[15]*Andalusian School of Public Health, Granada, Spain*
[16]*Public Health Department of Guipuzkoa, San Sebastian, Spain*
[17]*Public Health Institute of Navarra, Pamplona, Spain*
[18]*Epidemiology Department, Health Council of Murcia, Murcia, Spain*
[19]*Public Health and Health Planning Directorate, Asturias, Spain*
[20]*Cancer Epidemiology Unit, University of Oxford, Oxford, United Kingdom*
[21]*Department of Public Health and Primary Care, MRC Centre for Nutrition in Cancer Prevention and Survival, University of Cambridge, Cambridge, United Kingdom*
[22]*Clinical Gerontology Unit, University of Cambridge, Cambridge, United Kingdom*
[23]*German Institute of Human Nutrition, Potsdam-Rehbücke, Germany*
[24]*Division of Clinical Epidemiology, Deutsches Krebsforschungszentrum, Heidelberg, Germany*
[25]*Department of Clinical Epidemiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark*
[26]*Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark*
[27]*Department of Hygiene and Epidemiology, Medical School, University of Athens, Athens, Greece*
[28]*Department of Epidemiology, Catalan Institute of Oncology, Barcelona, Spain*
[29]*Nutrition and Hormones Group, International Agency for Research on Cancer, Lyon, France*
[30]*University of Florence, Florence, Italy*
[31]*Institute of Molecular Pathology and Immunology, Medical Faculty, University of Porto, Porto, Portugal*

***Helicobacter pylori* (*H. pylori*), atrophic gastritis, dietary and life-style factors have been associated with gastric cancer (GC). These factors have been evaluated in a large case–control study nested in the European Prospective Investigation into Cancer and Nutrition carried out in 9 countries, including the Mediterranean area. Participants, enrolled in 1992–1998, provided life-style and dietary information and a blood sample (360,000; mean follow-up: 6.1 years). For 233 GC cases diagnosed after enrolment and their 910 controls individually-matched by center, gender, age and blood donation date *H. pylori* antibodies (antilysate and antiCagA) and**

Abbreviations: CI, confidence intervals; EPIC, European Prospective Investigation into Cancer and Nutrition; GC, gastric cancer; GEJ, gastro esophageal junction; *H. pylori*, *Helicobacter pylori*; OR, odds ratio; PGA, pepsinogen A; SCAG, severe chronic atrophic gastritis.

Grant sponsor: European Commission; Grant number: QLG1-CT-2001-01049; Grant sponsor: Spanish Ministry of Health; Grant number: RCESP-C03/09; Grant sponsors: European Commission (SANCO); Italian Association for Research on Cancer (AIRC); Ligue contre le Cancer (France); Société 3M (France); Mutuelle Générale de l'Education Nationale; Institut National de la Santé et de la Recherche Médicale (INSERM); German Cancer Aid; German Cancer Research Center; German Federal Ministry of Education and Research; Danish Cancer Society; the participating regional governments and institutions of Spain; Cancer Research UK; Medical Research Council, UK; The Stroke Association, UK; British Heart Foundation; Department of Health, UK; Food Standards Agency, UK; the Wellcome Trust, UK; Greek Ministry of Health; Greek Ministry of Education; Dutch Ministry of Public Health, Welfare and Sports; Dutch Ministry of Health; Dutch Prevention Funds; LK Research Funds; Dutch ZON (Zorg Onderzoek Nederland); World Cancer Research Fund (WCRF); Swedish Cancer Society; Swedish Scientific Council; Regional Government of Skane, Sweden.

***Correspondence to:** Molecular and Nutritional Epidemiology Unit, CSPO–Scientific Institute of Tuscany, Via Cosimo il Vecchio 2, 50139 Florence, Italy. Fax: +39-055-32697829. E-mail: d.palli@cspo.it

plasma Pepsinogen A (PGA) were measured by ELISA methods. Severe chronic atrophic gastritis (SCAG) was defined as PGA circulating levels <22 μg/l. Overall, in a conditional logistic regression analysis adjusted for education, smoke, weight and consumption of total vegetables, fruit, red and preserved meat, *H. pylori* seropositivity was associated with GC risk. Subjects showing only antibodies anti-*H. pylori* lysate, however, were not at increased risk, while those with antiCagA antibodies had a 3.4-fold increased risk. Overall, the odds ratio associated with SCAG was 3.3 (95% CI 2.2–5.2). According to site, the risk of noncardia GC associated with CagA seropositivity showed a further increase (OR 6.5; 95% CI 3.3–12.6); on the other hand, a ten-fold increased risk of cardia GC was associated with SCAG (OR 11.0; 95% CI 3.0–40.9). These results support the causal relationship between *H. pylori* CagA+ strains infection, and GC in these European populations even after taking into account dietary habits. This association was limited to distal GC, while serologically defined SCAG was strongly associated with cardia GC, thus suggesting a divergent risk pattern for these 2 sites.

Key words: *Helicobacter pylori*; stomach cancer; epidemiology; pepsinogen; chronic atrophic gastritis

Despite a dramatic decline in incidence, gastric cancer (GC) remains the fourth more common cancer and the second lethal neoplasm worldwide.[1] Nutritional, infectious, and genetic factors have been shown to play a role in gastric carcinogenesis.[2–4] The relationship between *Helicobacter pylori* (*H. pylori*) infection and GC has been clarified in the first decade after its identification.[5] *H. pylori* has been classified by the International Agency for Research on Cancer (IARC) as a definite carcinogen to humans (Group 1).[3] *H. pylori* infection induces a chronic inflammation of the gastric mucosa that is intensified by the host inflammatory immune response with high levels of several cytokines. This chronic process leads, in a minority of infected individuals, to the development of GC through a series of intermediate progressive stages including mild and severe chronic gastritis, atrophic gastritis, gastric atrophy, characterized by hypochlorhydria, and intestinal metaplasia.[6–8] Recent meta-analyses have estimated that the infection increases the GC risk by 2- to 3-fold[9–11] with higher estimates for noncardia GC.

Up to 60% of the general population in most European areas is infected with *H. pylori*[12] and 90–100% in less developed countries. It is clear that GC occurs only in a minority of infected individuals. Different outcomes of the infection have been related to variations of *H. pylori* pathogenicity, host susceptibility, environmental and life-style factors (particularly diet and age at infection) and interactions among all these factors.[13] There is evidence that different strains of *H. pylori* may play a role in gastric carcinogenesis and subjects infected with cytotoxin CagA positive *H. pylori* strains (*H. pylori* CagA+) are at increased risk to develop GC in comparison with subjects infected with *H. pylori* CagA− strains.[14–16] Also, only CagA positive strains were able to induce GC in experimental infected Mongolian gerbils.[17] In a recent meta-analysis based on 16 case–control studies with age- and sex-matched controls, the infection with CagA positive strains was associated with an ~2-fold increased risk of noncardia GC among *H. pylori*-infected subjects.[18] This could be related to the active injection of CagA into the gastric epithelial cell through the Type IV secretion apparatus encoded by the cag pathogenicity island.[19] The tyrosine phosphorylation of CagA and its interaction with membrane proteins would trigger a cascade of signal transductions leading to the induction of chronic inflammatory responses (*e.g.* through the production of IL-8) that would in turn favor the neoplastic transformation.[20–22] Recently, it has also been reported that CagA is able to induce a transition from polarized epithelial cells (showing cell–cell adhesion) to a more invasive phenotype.[23]

Severe chronic atrophic gastritis (SCAG) is considered as a predisposing factor for GC, particularly of the intestinal type. Pepsinogen is secreted as 2 biochemical distinct groups of isoenzymes: Pepsinogen A (PGA) and Pepsinogen C. Pepsinogen C is secreted by corpus chief cells, antral glands and duodenal bulb, and PGA is secreted only by the chief cells of the gastric corpus and the fundus, and its serum level decreases with an increasing grade of corpus atrophy.[24,25] Traditionally, low levels of PGA have been used to identify SCAG in the frame of epidemiological projects or ''screening'' programs.[26–28] *H. pylori* infection is recognized as the main determinant of this precancerous lesion, although once that SCAG is established, the bacterium is less likely to survive and antibodies may be lost over time.

Our large case–control study nested in a cohort of 360,000 adults from several European countries (including the Mediterranean area), all with a blood sample donated at enrolment, offers the opportunity to evaluate the association between *H. pylori* infection and GC risk in a prospective design taking into account the effect of serologically defined SCAG and the role of dietary and other environmental factors in gastric carcinogenesis.

Material and methods

The EPIC cohort

EPIC is a multicenter prospective study coordinated by the IARC (International Agency on Research on Cancer, Lyon, France) aimed to study the association between diet and life-style habits and cancer, based on healthy adults who voluntarily agreed to participate in the study and to have their health status followed up. The enrolment started in 1992–1993 and was completed in 1998 in 23 collaborating centers in 10 European countries (Sweden, Denmark, Norway, Netherlands, UK, France, Germany, Spain, Italy and Greece). Overall ~520,000 subjects (aged 35–74) of both genders have been recruited and blood samples at enrolment were obtained from over 380,000 subjects.

The study design has been reported in full elsewhere.[29] Briefly, the volunteers were mostly enrolled from the general population residing in a specific area. Exceptions were the French cohort, based on female members of a national health insurance for school teachers and the Utrecht cohort (Netherlands), based on women attending the local breast cancer screening program. The 5 Spanish cohorts and 2 Italian cohorts (Ragusa and Turin) were mostly based on blood donors; most of the Oxford cohort in the UK was based on vegetarian and health conscious volunteers recruited across the whole country.

Detailed information on the consumption of a series of foods and drinks has been collected by country-specific food frequency questionnaires, validated in a pilot phase. For calibrating dietary measurements across countries and different questionnaires and to correct for systematic over- or under-estimation of dietary intakes,[30] a 24-hr dietary recall has been obtained from a random sample (8%) of the cohort (36,994 participants).[31] Detailed information has also been collected on education and other socioeconomic variables, smoking and alcohol drinking history, physical activity, reproductive history and medical history by a common life-style questionnaire developed in 2 separate versions for males and females. Anthropometric measurements (including weight and height) have been collected according to a standard protocol.

The EPIC cohort has been followed up since inception through population based cancer registries or other available sources as pathology hospital registries, health insurance records and active follow-up through participants and their next-of-kin. Mortality data were also obtained from mortality register at the regional or national level.

Design of the nested case–control study (EPIC-EURGAST)

GC cases. All newly diagnosed GC cases (ICD 10 C.16) identified in 9 national cohorts (except Norway, for a total of ~225,000 women and 135,000 men) after the date of recruitment until the end of follow-up (December 1999 or September 2002 depending on the Centre), for which a blood sample was available,

were included. All cases were histologically confirmed as adenocarcinomas. GC incidence rates in the different cohorts agreed with rates provided by local registries (Ferrari P, manuscript in preparation) and the male/female ratio was 2:1 as expected.

Matched controls. For each case, 4 controls, individually matched by centre, gender, age (±2.5 years) and blood donation date (±45 days), were randomly selected among those still at risk of GC (at the time of diagnosis of each case).

Blood samples. All individual available plasma samples were retrieved from the collaborative Biobank of the EPIC project (IARC, Lyon) or from local banks in Denmark and Sweden, aliquoted and shipped on dry ice to the study laboratories. Assays were performed blindly regarding the case–control status or any individual information.

Laboratory assays

*Quantitation of anti-*H. pylori *and antiCagA IgG antibodies.* For quantitation of anti-*H. pylori* IgG antibodies, ninety-six-well, flat-bottomed microtiter plates (Nunc, Roskilde, Denmark) were coated with *H. pylori* lysate (CCUG strain) prepared as already described in detail elsewhere.[32] Hundred microliters of lysate at 1 μg/ml in PBS pH 7.4 were used and coating was allowed to proceed for 2 hr at room temperature. After 3 washes with PBS containing 0.05% (v/v) Tween-20, the plates were incubated with 250 μl/well of a solution of polyvinylpyrrolidone-15 (Serva, Heidelberg, Germany) for 1 hr at room temperature. After further washes, doubling dilutions of plasma samples (starting from 1:200), diluted in PBS-Tween-20 containing 2% (w/v) BSA (Sigma Chemical, St. Louis, MO) were added to the plates and incubation was allowed to proceed for 1 hr at room temperature. After extensive washing with PBS-Tween-20, bound IgG antibodies were quantified by the addition of an alkaline phosphatase-conjugated polyclonal affinity-purified goat antihuman IgG (Sigma Chemical), diluted 1:10,000 in PBS-Tween-20-BSA and incubated for 3 hr at 30°C. The plates were then washed, and 100 μl of *p*-nitrophenylphosphate (Sigma Chemical) at 1 mg/ml in diethanolamine buffer were added to each well. The chromogenic reaction was allowed to proceed for 1 hr in the dark at room temperature and was then stopped by the addition of 4 N NaOH. Optical densities were read at 405 and 650 nm. Antigen-specific IgG antibody titres were expressed as ELISA Units (EU), and were determined by interpolation relative to a standard curve constructed by a serial dilution of a standard positive control, which consisted of a pool of samples from subjects known to be positive for *H. pylori* infection and having antibodies as determined by Western blotting analyses. A cut-off value of 100 EU was defined using samples from individuals negative for *H. pylori* infection as determined by urea breath test, and by microbiological and other serological assays (Western blotting). Plasma samples giving EU values above 100 were considered as positive for anti-*H. pylori* IgG antibodies. In previous experiments, this assay exhibited specificity and sensitivity higher than 90% (Berti D and Del Giudice G, unpublished data).

For detection and quantitation of antiCagA IgG antibodies, ninety-six-well, flat-bottomed microtiter plates (Nunc) were coated with 100 μl of full-length recombinant CagA (lot 010101-CAG07/28) at the concentration of 0.9 μg/ml in PBS pH 7.4. Coating was allowed to proceed for 1.5 hr at 30°C. After 3 washes with PBS containing 0.05% (v/v) Tween-20, the plates were incubated with 250 μl/well of a solution of polyvinylpyrrolidone-15 (Serva) for 1.5 hr at room temperature. After further washes, doubling dilutions of plasma samples (starting from 1:400), diluted in PBS-Tween-20 containing 2% (w/v) BSA (Sigma Chemical) were added to the plates. Antibodies were allowed to react with the solid-phase antigens for 1 hr at 37°C. After extensive washing with PBS-Tween-20, bound IgG antibodies were quantified by the addition of an alkaline phosphatase-conjugated polyclonal affinity-purified goat antihuman IgG (Sigma Chemical), diluted 1:2,000 in PBS-Tween-20-BSA and incubated for 3 hr at room temperature. The plates were then washed, and 100 μl of *p*-nitrophenylphosphate (Sigma Chemical) at 1 mg/ml in diethanolamine buffer were added to each well. The chromogenic reaction was allowed to proceed for 40 min in the dark at room temperature and was then stopped by the addition of 4 N NaOH. Optical densities were read at 405 and 650 nm. Antigen-specific IgG antibody titres were expressed as ELISA Units (EU), and were determined by interpolation relative to a standard curve constructed by a serial dilution of a standard positive control. A cut-off value of 30 EU was defined using samples from individuals negative for *H. pylori* infection as determined by urea breath test, and by microbiological and by other serological assays (Western blotting). Plasma samples giving EU values above 30 were considered as positive for antiCagA IgG antibodies.

Determination of plasma pepsinogen A. Pepsinogen A (PGA) was assayed in the plasma samples by means of microplate-based quantitative enzyme immunoassay (ELISA) using a commercial kits (Biohit, Helsinki, Finland). The assay is based on a sandwich enzyme immunoassay technique with PGA-specific capture monoclonal antibodies adsorbed on a microwell plate and detection antibodies labeled with horseradish peroxidase. The concentrations of PGA in unknown samples were calculated on a calibration curve, made by using 3 calibrators (human serum samples with lot-specific PGA values) provided by the manufacturer. The coefficients of variations (intra- and inter-assay) did not exceed 5%. The reference range, calculated on a large series of control subjects, was 22–82 μg/l.[24]

Gastric cancer case definition. A panel of experienced pathologists met to re-evaluate GC diagnoses based on the material collected in each center for all GC identified. Original diagnostic reports were obtained from local pathology departments, translated and reviewed together with available H&E-stained slides. The Lauren classification was used for the histological type of GC. Briefly, among 233 GC cases, 91 (39.0%) were classified as intestinal adenocarcinoma, 89 (38.2%) as diffuse adenocarcinoma, 35 (15.0%) as adenocarcinoma NOS, 12 (5.2%) were unclassified adenocarcinomas, 2 (0.9%) were mixed adenocarcinomas and 4 (1.7%) gastric stumps.

According to the site within the stomach, 127 (54.5%) cases were classified as noncardia, 54 (23.2%) as cardia and 4 were considered mixed; for 44 cases the site was undetermined. An additional group of 16 cases were classified into a specific sub-group (gastro-oesophageal junction) but were not included in the main analyses.

Overall, the presence of antibodies to *H. pylori* lysate and full length recombinant CagA have been assessed for 233 cases and their 910 matched controls. Most serological assays were carried out for all the eligible 4 matched controls; for 12 cases, however, results were available only for 3 matched controls, 2 cases had only 2 matched controls and 2 cases had only 1 matched control. PGA measurements, on the other hand, were available for 231 cases and their 901 matched controls.

Statistical analysis

Overall *H. pylori* seropositivity was defined as the presence of at least 1 type of 2 specific antibodies (anti-*H. pylori* lysate and antiCagA IG antibodies); the latter results were used to create additional variables, as 1 term for each specific antibody or specific combinations in order to identify mutually exclusive subgroups among seropositive subjects (*H. pylori* seropositive but CagA negative *vs.* CagA positive). In addition, SCAG was serologically defined as a PGA circulating level lower than 22 μg/l.

Odds ratios (OR) for the association of *H. pylori* seropositivity or specific antiCagA antibodies with GC were calculated by multiple conditional logistic regression models. Confidence intervals (95% CI) were computed using the standard errors of the pertinent regression coefficients. The effects of additional potential confounders (other than the matching factors controlled for by design) like tobacco smoking, socio-economic level as defined by educa-

TABLE I – DISTRIBUTION OF 233 GASTRIC CANCER CASES AND 910 MATCHED CONTROLS, PREVALENCE OF *H.PYLORI* SEROPOSITIVITY, SEROPREVALENCE OF ANTIBODIES ANTI-CagA AND PREVALENCE OF SEVERE CHRONIC ATROPHIC GASTRITIS (SCAG) (SEROLOGICALLY DEFINED, PEPSINOGEN A <22 μg/l) AT ENROLMENT, ACCORDING TO GENDER, AGE, COUNTRY AND BROAD GEOGRAPHICAL AREA (EPIC-EURGAST 1993–2002)

	GC Cases (*n*)	Controls (*n*)	*H. pylori*[1] seroprevalence				*H. pylori* CagA seroprevalence				Severe chronic atrophic gastritis			
			Cases		Controls		Cases		Controls		Cases		Controls	
			n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Gender														
Males	127	490	104	81.9	333	68.0	90	70.9	234	47.8	23	18.3	38	7.8
Females	106	420	91	85.9	292	69.5	74	69.8	186	44.3	26	24.8	35	8.4
Age														
<60 years	113	425	90	79.7	271	63.8	80	70.8	178	41.9	13	11.7	31	7.4
≥60 years	120	485	105	87.5	354	73.0	84	70.0	242	49.9	36	30.0	42	8.8
Mediterranean countries														
Italy	43	170	40	93.0	108	63.5	31	72.1	69	40.6	11	25.6	19	11.5
Spain	27	107	26	96.3	96	89.7	22	81.5	59	55.1	6	22.2	11	10.3
Greece	12	48	11	91.7	45	93.8	10	83.3	38	79.2	1	8.3	3	6.4
Total	*82*	*325*	*77*	*93.9*	*249*	*76.6*	*63*	*76.8*	*166*	*51.1*	*18*	*22.0*	*33*	*10.3*
Central-Northern Europe														
France	3	12	2	66.7	4	33.3	2	66.7	1	8.3	–	–	–	–
UK	19	75	14	73.7	45	60.0	13	68.4	32	42.7	6	31.6	7	9.3
The Netherlands	19	76	16	84.2	52	68.4	11	57.9	30	39.5	4	21.1	2	2.7
Germany	31	124	27	87.1	77	74.0	22	71.0	48	38.7	7	23.3	11	8.9
Sweden	55	213	47	85.5	151	70.9	45	81.8	122	57.3	13	24.1	14	6.6
Denmark	24	85	12	50.0	47	55.3	8	33.3	21	24.7	1	4.2	6	7.1
Total	*151*	*585*	*118*	*78.2*	*376*	*64.3*	*101*	*66.9*	*254*	*43.4*	*31*	*20.8*	*40*	*6.9*
Total	*233*	*910*	*195*	*83.7*	*625*	*68.7*	*164*	*70.4*	*420*	*46.2*	*49*	*21.2*	*73*	*8.1*

[1]Defined as presence of antibodies anti-*H. pylori* lysate and antiCagA.

tional level, were examined by including in the logistic regression models terms for education (low/high), smoking history (current-/ex-/never-smoker) and weight. Adjustments for dietary variables were also performed adding to the models terms for selected food groups that were considered as risk or protective factors (red and preserved meat; total vegetables and fruit), using the predicted (calibrated) values for each individual on a continuous scale.[30] The effects of *H. pylori* infection, CagA seropositivity and SCAG status were also evaluated adding the terms for *H. pylori* seropositivity (or CagA seropositivity) and for presence/absence of SCAG in the same model. Separate analyses were carried out according to gender, age classes (<60 years and ≥60 years), geographical area (countries in Central-Northern and Southern Europe), site of the tumor (cardia and non-cardia) and histotypes according to Lauren classification (intestinal and diffuse type). For the 2 latter sub-group analyses, the 2 main categories did not add to the total because of unclassified GC cases. The 2 broad geographical areas were defined *a priori*. We tested also a series of interactions between *H. pylori* seropositivity, CagA seropositivity and SCAG status and age and geographical area. All analyses were performed using the SAS statistical software (SAS/STAT version 9.1.3); a p-value <0.05 was considered statistically significant.

Results

After an average follow-up of 6.1 years, 233 GC cases were identified (127 males) and with results available from laboratory assays for the analyses; among them, 54 (36 males) were classified as carcinomas of the gastric cardia. Eighty-two cases were identified in the 3 Mediterranean cohorts (Italy, Spain and Greece) and 151 in the 6 Central-Northern Europe cohorts (France, UK, The Netherlands, Germany, Sweden and Denmark) (Table I).

H. pylori *seropositivity*

Overall, the prevalence of *H. pylori* seropositivity was 83.7% (195/233) among cases and 68.7 (625/910) among their matched controls, being higher among subjects older than 60 years and in Mediterranean countries (where 93.9% of cases and 76.6% of controls were seropositive).

The prevalence of specific antiCagA antibodies was 70.4% (164/233) among cases and 46.2% (420/910) among controls. The CagA seroprevalence tended to be higher in older controls and in those from Mediterranean cohorts (Table I). A small group of study subjects showed detectable levels of antiCagA antibodies (1.7 and 3.9% respectively, among cases and controls), while their specific antilysate antibodies were below the threshold value. Overall, the proportion of *H. pylori* seropositive subjects showing antiCagA antibodies was 84.1% (164/195) among cases and 67.2% (420/625) among controls with limited variation across the national cohorts.

The prevalence of *H. pylori* and CagA seropositivity among cardia GC cases was 64.8% (35/54) and 46.3% (25/54), and among noncardia GC cases 90.6% (115/127) and 78.7% (100/127), respectively.

Serologically defined SCAG

The prevalence of serologically defined SCAG was 21.2% among cases (49/231) and 8.1% (73/901) among controls (Table I). Again, some differences were evident among controls across different countries, with a higher prevalence in the Mediterranean cohorts. On the other hand, the prevalence of SCAG among GC cases was very similar in the 2 broad geographical areas. When SCAG prevalence was evaluated according to the presence of anti-*H. pylori* antibodies, we found that SCAG prevalence was 23.3% (45/193) and 10.5% (4/38) respectively, among *H. pylori* seropositive and seronegative GC cases ($p = 0.08$). SCAG prevalence was 9.1% (56/616) among *H. pylori* seropositive controls and 6.0% (17/285) among *H. pylori* negative controls ($p = 0.11$). According to antiCagA status, SCAG prevalence was 23.5% (38/162) and 15.9% (11/69) respectively among CagA seropositive and seronegative GC cases ($p = 0.20$), and 9.4% (39/414) and 7.0% (34/487) respectively among CagA seropositive and seronegative controls ($p = 0.22$).

H. pylori *lysate and CagA seropositivity and GC risk*

Overall, in a conditional multivariate model taking into account terms for education, smoking history, weight, and calibrated average daily consumption of total vegetables, total fruit, red and pre-

TABLE II – ASSOCIATION OF *H. PYLORI* SEROPOSITIVITY, AND SEROLOGICALLY DETERMINED SEVERE CHRONIC ATROPHIC GASTRITIS (SCAG) WITH GC RISK BASED ON SEPARATE MODELS: OVERALL, BY SITE (NONCARDIA AND CARDIA) AND BY HISTOTYPE (DIFFUSE AND INTESTINAL). NESTED CASE–CONTROL STUDY IN THE EPIC COHORTS (EACH MATCHED SET WITH 1 GC CASE AND AGE, SEX, BLOOD DONATION DATE AND CENTER MATCHED CONTROLS) (EPIC-EURGAST 1993–2002)

Variable(s) of interest in each separate model	GC (n = 233)		Noncardia (n = 127)		Cardia (n = 54)		Diffuse type (n = 89)		Intestinal type (n = 91)	
	OR[1]	95% CI[1]	OR[1]	95% CI[1]	OR[1]	95% CI[1]	OR[1]	95% CI[1]	OR[1]	95% CI[1]
Antibodies anti-*H.pylori* (yes/no)	2.6	1.7–3.9	4.7	2.5–9.0	0.8	0.4–1.8	4.2	1.9–9.2	2.6	1.4–4.9
Only antibodies anti-*H.pylori* lysate (yes/no)	1.2	0.7–2.0	1.6	0.7–3.8	0.8	0.3–2.1	2.0	0.8–5.2	1.1	0.5–2.8
Antibodies antiCagA (yes/no)	3.4	2.2–5.2	6.5	3.3–12.6	0.8	0.4–1.9	5.8	2.5–13.3	3.2	1.7–6.1
SCAG (yes/no)	3.4	2.2–5.2	2.4	1.3–4.5	11.0	3.0–40.9	1.6	0.7–3.5	4.8	2.3–10.0
Antibodies anti-*H.pylori* (yes/no)	2.6	1.7–3.9	5.1	2.6–9.8	0.7	0.3–1.6	4.1	1.8–9.1	2.5	1.3–4.7
SCAG (yes/no)	3.3	2.1–5.2	2.7	1.5–5.1	11.9	3.1–45.6	1.7	0.7–3.8	4.5	2.2–9.4
Antibodies antiCagA (yes/no)	3.1	2.2–4.4	5.4	3.2–9.1	0.9	0.5–2.0	4.0	2.2–7.4	2.8	1.6–4.7
SCAG (yes/no)	3.3	2.1–5.2	2.6	1.3–4.9	11.0	2.9–40.7	1.7	0.7–3.9	4.2	2.0–9.1

[1]Odds ratios (OR) and 95% confidence intervals (CI) estimated by a series of conditional logistic models including terms for education (low/high), smoking history (never-/ex-/current-smoker), weight (in continuous), total vegetables, fruit, red and preserved meat (calibrated values, in continuous) and the variable(s) of interest as indicated in each panel.

served meat, *H. pylori* seropositivity was associated with an approximate 2.5-fold risk of developing gastric cancer (OR 2.6; 95% CI 1.7–3.9) (Table II).

When we included in the same model a specific term for those subjects showing only antilysate antibodies (that is *H. pylori* lysate seropositive but CagA seronegative subjects) and 1 for those showing antiCagA antibodies (independently from the antilysate antibodies status), results showed that the first group was not at increased risk, while CagA seropositive subjects showed an over 3-fold increased risk (OR 3.4; 95% CI 2.2–5.2).

When we restricted the analyses to noncardia GC (n = 127), the association with overall *H. pylori* seropositivity was more evident; on the other hand, a 6-fold increased risk emerged with CagA seropositivity (OR 6.5; 95% CI 3.3–12.6).

In the sub-group of cardia GC (n = 54) no association with *H. pylori* seropositivity was found overall and when *H. pylori* lysate and CagA were included separately.

In analyses carried out according to gastric cancer histotypes, *H. pylori* seropositivity was positively associated with both diffuse and intestinal type. When we included separately the terms for *H. pylori* lysate and CagA seropositivity in the model, the association with CagA seropositivity tended to be stronger for the diffuse than for the intestinal type.

No differences in the association between GC risk and *H. pylori* seropositivity emerged according to gender and age (Fig. 1). The effect of CagA tended to be stronger in younger subjects (Fig. 2).

In the Mediterranean cohorts, *H. pylori* seropositivity was overall associated with a 7.4-fold increase in GC risk; in the Central-Northern European cohorts the increase in GC risk among seropositive subjects was limited approximately to a 2-fold increase (Fig. 1).

When we considered the specific effects of *H. pylori* lysate and CagA seropositivity by geographical area, the association with GC risk appeared to be limited to antiCagA antibodies in the Central-Northern cohorts. In the Mediterranean cohorts, both *H. pylori* lysate and CagA seropositivity were associated with GC risk, respectively a 4-fold (OR 4.0; 95% CI 1.2–13.2, data not shown) and a 9-fold increase in comparison to seronegative subjects (Fig. 2).

Furthermore, specific models were developed to evaluate the effect of antiCagA antibodies among all *H. pylori* seropositive subjects; the results confirmed in the overall GC series a strongly increased risk for subjects with antiCagA antibodies in respect to *H. pylori* seropositive but CagA negative subjects (OR 3.0; 95% CI 1.9–4.6). This effect was particularly evident in younger subjects (OR 6.2; 95% CI 2.7–13.8).

Serologically defined SCAG and GC risk

A positive association between SCAG and gastric cancer risk was evident in the whole series (OR 3.4; 95% CI 2.2–5.2). This did not materially change when the term for *H. pylori* seropositivity was added to the model (Table II). Overall, the association with SCAG tended to be more evident in older subjects, while no differences emerged by gender (Fig. 3).

In the Central-Northern European cohorts, the strongest association with GC risk was actually shown by serologically defined SCAG (OR 4.2; 95% CI 2.3–7.7), that persisted substantially unchanged also in analyses taking into account CagA seropositivity. In the Mediterranean cohorts, the association with SCAG tended to be weaker (Fig. 3).

The risk of noncardia GC associated with SCAG was 2.4-fold increased (Table II). The results did not change when the other 48 GC cases of undetermined or mixed site were also considered in this category.

On the other hand, the presence of SCAG was associated with a ten-fold increased cardia GC risk (OR 11.0; 95% CI 3.0–40.9). This association persisted also when the separate terms for antilysate and antiCagA antibodies were added to the model (Table II).

According to histological subtypes, the association between GC risk and SCAG was statistically significant only for the intestinal type (Table II).

Interactions

Statistically significant interactions in modifying the risk to develop GC were found between 2 pairs of variables: *H. pylori* overall seropositivity and being enrolled in a Mediterranean cohort (p = 0.04) as well as the presence of a serologically defined SCAG and being older than 60 years old at enrolment (p = 0.015). When we examined the association of noncardia GC risk in the 2 different areas, these differences were much less evident, probably due to the lower proportion of cardia cases in the Mediterranean countries (12% in the Mediterranean area *vs.* 29% in Northern Central Europe). Also interactions between the 2 areas and specific antibodies (only antilysate *vs.* antiCagA) did not reach the level of statistical significance.

All analyses have also been repeated excluding GC cases diagnosed in the first year after the date of enrolment (n = 32), but results did not change.

We also repeated the analyses in 2 separate time periods (below and above the median latency between enrolment and diagnosis, ~3 years), but the results were quite similar with no suggestion of further risk increase with increasing duration of follow-up (data not shown).

Discussion

In this nested case–control study carried out in the frame of a specific project within the European Prospective Investigation into

		N.	OR (95% CI)
	All matched sets	233	2.6 (1.7-3.9)
Sex	Males	127	2.4 (1.4-4.1)
	Females	106	3.0 (1.6-5.6)
Age	<60 years	113	3.0 (1.7-5.4)
	≥60 years	120	2.8 (1.5-5.5)
Area	Mediterranean	82	7.4 (2.5-21.5)
	Centr.North.Eur.	151	2.0 (1.3-3.1)
Site	Cardia	54	0.8 (0.4-1.8)
	Non-cardia	127	4.7 (2.5-9.0)
Histological	Diffuse	89	4.2 (1.9-9.2)
type	Intestinal	91	2.6 (1.4-4.9)

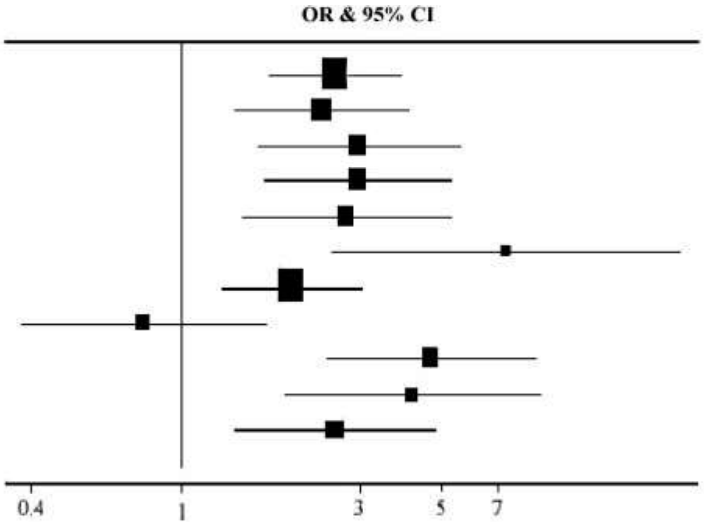

Figure 1 – Estimates of gastric cancer risk associated with *H. pylori* seropositivity (antibodies anti-*H. pylori* lysate and antiCagA) overall and by sex, age group, area, site and histological type (EPIC-EURGAST 1993–2002). Odds ratios (OR) and 95% confidence intervals (CI) estimated by a series of conditional logistic models including terms for education (low/high), smoking history (never-/ex-/current-smoker) weight (in continuous), total vegetables, fruit, red and preserved meat (calibrated values, in continuous).

		N.	OR (95% CI)
	All matched sets	233	3.4 (2.2-5.2)
Sex	Males	127	3.0 (1.7-5.1)
	Females	106	4.3 (2.2-8.3)
Age	<60 years	113	5.7 (2.5-8.8)
	≥60 years	120	3.4 (1.7-6.6)
Area	Mediterranean	82	9.2 (3.1-27.5)
	Centr.North.Eur.	151	2.6 (1.7-4.2)
Site	Cardia	54	0.8 (0.4-1.9)
	Non-cardia	127	6.5 (3.3-13.6)
Histological	Diffuse	89	5.8 (2.5-13.3)
type	Intestinal	91	3.2 (1.7-6.1)

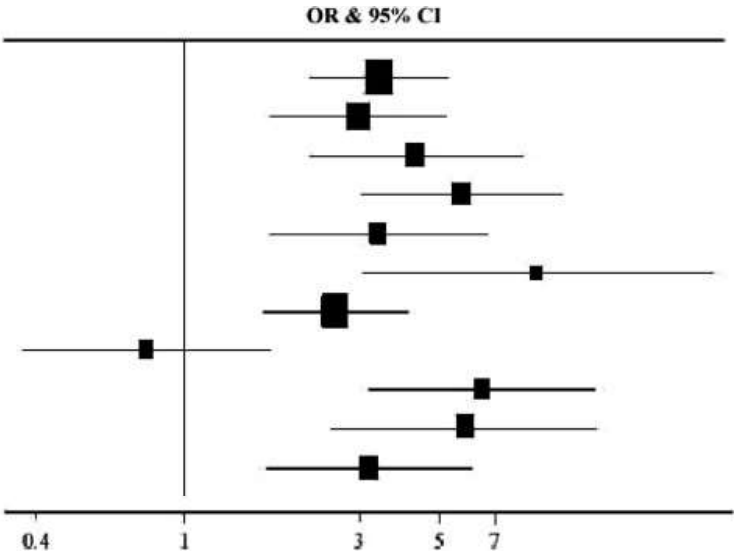

Figure 2 – Estimates of gastric cancer risk associated with *H. pylori* CagA seropositivity (antibodies antiCagA) overall and by sex, area, age group, site and histological type (EPIC-EURGAST 1993–2002). Odds ratios (OR) and 95% confidence intervals (CI) estimated by a series of conditional logistic models including terms for education (low/high), smoking history (never-/ex-/current-smoker) weight (in continuous) total vegetables, fruit, red and preserved meat (calibrated values, in continuous) and the terms for *H. pylori* lysate seropositivity (yes/no) and CagA seropositivity (yes/no).

Cancer and Nutrition (EPIC-EURGAST), we have found that the presence of specific antibodies against *H. pylori* CagA was associated with an over 3-fold increased risk of developing gastric cancer. Overall, GC risk did not appear to be increased for subjects showing only antibodies against *H. pylori* lysate. These results were based on an average follow-up period of 6 years and were obtained taking into account the dietary habits reported at enrolment. These results were even more evident in the subgroup of noncardia GC cases, while, on the other hand, there was no evidence of any association between cardia GC risk and *H. pylori* or CagA seropositivity.

In addition, the presence of a serologically defined severe chronic atrophic gastritis (Pepsinogen A serum level lower than 22 μg/l) was overall associated with an increased GC risk of more than 3-fold, particularly among older individuals. This association persisted also after adjustment for *H. pylori* or CagA seropositivity. A specific and strong association of SCAG with GC of the cardia emerged, thus suggesting a possible divergent risk pattern for distal and cardia GC.

These results have been obtained taking into account a series of dietary determinants as consumption of total vegetables, fruit, red meat and preserved meat[33,34] in a large study population including also for the first time cohorts from Mediterranean countries.

The risk in *H. pylori* positive subjects we have found is quite similar to the recent estimate by a pooled analysis of 12 nested case–control studies within prospective cohorts[9] and slightly

		N.	OR (95% CI)
	All matched sets	233	3.4 (2.2-5.2)
Sex	Males	127	2.8 (1.5-5.3)
	Females	106	4.1 (2.2-7.6)
Age	<60 years	113	2.1 (1.0-4.7)
	≥60 years	120	5.2 (2.8-9.8)
Area	Mediterranean	82	2.5 (1.2-5.2)
	Centr.North.Eur.	151	4.2 (2.3-7.7)
Site	Cardia	54	11.0 (3.0-40.9)
	Non-cardia	127	2.4 (1.3-4.5)
Histological	Diffuse	89	1.6 (0.7-3.5)
type	Intestinal	91	4.8 (2.3-10.0)

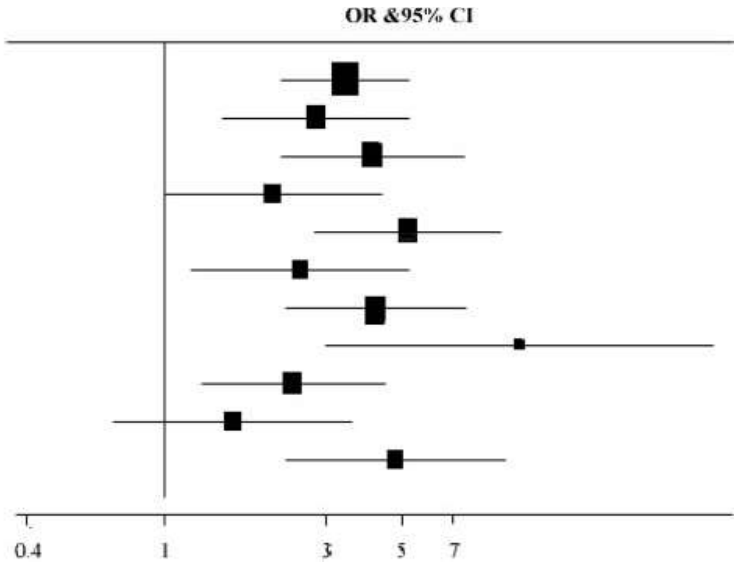

Figure 3 – Estimates of gastric cancer risk associated with serologically defined severe chronic atrophic gastritis (pepsinogen A <22 μg/l) overall and by sex, area, age group, site and histological type (EPIC-EURGAST 1993–2002). Odds ratios (OR) and 95% confidence intervals (CI) estimated by a series of conditional logistic models including terms for education (low/high), smoking history (never-/ex-/current-smoker) weight (in continuous) total vegetables, fruit, red and preserved meat (calibrated values, in continuous).

higher than estimates from meta-analyses considering both case–control and cohort studies limited to studies in which *H. pylori* infection was determined by serological assays[11] or more comprehensive.[10]

Several studies, both in animals and in humans, have suggested that the infection with *H. pylori* CagA positive strains increases the risk of GC over the risk associated with *H. pylori* infection alone and experimental studies have suggested the molecular basis for the pathological action of CagA.[19,22] Our result of an over 3-fold increased risk of GC associated with the presence of anti-CagA antibodies in the present nested case–control study is consistent with the results of a recent meta-analysis addressing this issue.[18] Our data showed very clearly that the risk of developing GC among *H. pylori* seropositive subjects was mostly related to an infection with *H. pylori* CagA+ strains.

A statistically significant association with the presence of anti-*H. pylori* lysate antibodies was evident only in a sub-group analysis for the Mediterranean cohorts, although a specific interaction did not emerge. Possible explanations for this finding (apart from chance) might be that in our cohorts enrolled in the Mediterranean countries the seroprevalence of *H. pylori* infection at baseline (1993–1998) appeared higher than in Northern-Central European cohorts, suggesting that the circulation of the bacterium might have been higher until recently. Our test to detect antibodies to *H. pylori* lysate was based on crude antigens from a specific strain (in contrast to the antiCagA test based on a full-length recombinant molecule) and this might also contribute to some variation in the ability to detect titres in different populations exposed to other strains.

As expected by the more frequently distal (noncardia) localization of the gastric pathology induced by the infection with *H. pylori*, when we considered separately cardia and noncardia GC cases, it was evident that the association between *H. pylori* CagA seropositivity and GC risk was confined to noncardia GC cases with a 6-fold increased risk.

On the other hand, the presence of serologically defined SCAG was overall associated with a 3-fold increased risk of GC. PGA is a marker of corpus atrophy and SCAG tended to be more clearly associated with GC in Northern Europe than in the Mediterranean countries. This may suggest that factors related to Pernicious anemia might play a role in noncardia GC associated with *H. pylori* infection.[35]

Our result of a strong association between serologically defined SCAG and adenocarcinoma of the gastric cardia is in agreement with the results of a recently published population-based case–control study carried out in Sweden.[36] In the same study, no association was evident between cardia GC and *H. pylori* seropositivity. A number of studies reported a lack of association or a negative association between *H. pylori* and cardia GC[37–39] with a few exceptions.[40] Such association was not observed in a meta-analysis based on 42 studies (both case–control and cohort studies)[10] and in a recent study with an extended follow-up period.[41] In general, however, the anatomical site of cardia remains poorly defined and a number of studies may have been unable to differentiate between anatomical regions. In our study, all diagnoses have been revised by a panel of pathologists; in addition, we also performed an analysis taking into account 16 cases that had been defined as adenocarcinomas of the GEJ and results were weaker based on this less clear-cut definition of cardia GC. A complex interplay between gastro-oesophageal reflux disease and intestinal metaplasia has been recently postulated for cardia cancer[42]; our results would support a role for cardia metaplasia, a long term consequence of *H. pylori* infection possibly leading, over time and in presence of SCAG, to a reduction of antibody titres and seroreversion in a proportion of subjects.

The different proportion of cardia and noncardia GC could contribute to explain the geographical differences in the estimates of the association between *H. pylori* seropositivity and GC that resulted higher in Mediterranean countries, where the proportion of cardia cases was lower than in Northern-Central Europe. A difference in the proportion of cardia and noncardia GC cases was also evident among subjects of different ages.

Diffuse gastric adenocarcinoma appeared to be more associated with *H. pylori* CagA seropositivity than the intestinal type, while the association with SCAG appeared more evident for the latter histological type. This stronger association between *H. pylori* seropositivity and diffuse GC was confirmed when we restricted the analysis to noncardia GC cases. On the other hand, the strong association between SCAG and intestinal adenocarcinoma persisted, even if less evident, in the sub-group of noncardia carcino-

mas, while in the cardia cases, no diffuse adenocarcinoma case occurred among subjects with serologically defined SCAG.

Our study design had several advantages. Its prospective nature implicates that blood samples used for *H. pylori* serology and Pepsinogen A determination were collected well before the development of the disease, thus reducing the misclassification due to the loss of anti-*H. pylori* antibodies once SCAG is established. A relevant point in assessing the magnitude of the association between *H. pylori* and GC is the possible attenuation of risk when *H. pylori* infection is assessed closer to the time of cancer diagnosis.[9] We evaluated the association between GC and *H. pylori* seropositivity according to length of follow-up (below and above 3 years of follow-up) and results did not suggest differences in risk, although one has to consider that our follow-up is relatively short. An accurate ascertainment of disease through cancer registries and clinical records has been performed, and all cases were histologically confirmed. Dietary and life-style variables, used as adjusting variables, were collected through validated questionnaires and calibration of dietary variables allowed to reduce the effect of systematic over- or underestimation of dietary intakes. The sample size of our study, however, was relatively modest and most subgroup analyses had limited power, showing overlapping results.

In conclusion, our results confirm a causal role of *H. pylori* infection in the pathogenesis of GC also after taking into account the dietary habits reported at baseline. Our results have also shown that CagA positive strains play a crucial role in noncardia gastric carcinogenesis, while SCAG results associated with a strongly increased risk of cardia cancer. Although a long term *H. pylori* infection is the main cause of SCAG, our findings might suggest 2 divergent risk patterns for noncardia (*i.e.* distal) and cardia gastric cancer.

Acknowledgements

The authors thank all study participants for their availability. We thank Dr. Daniele Casini (Research Center, Novartis Vaccines, Siena, Italy) and Mr. Adriano Tasinato (University Hospital, Padua, Italy) for technical support for *H. pylori* serology and plasma Pepsinogens determinations. The authors also thank the other members of the Panel of Pathologists for their valuable work: Dr. Roger Stenling, Umea, Sweden; Dr. U Mahlke, Postdam, Germany; Dr. Hendrik Bläker, Heildelberg; Germany; Dr. Vicki Save, Cambridge, United Kingdom; Dr. Claus Fenger, Copenhagen, Denmark; Dr. Julio Torrado, San Sebastian, Spain; Dr. Johan Offerhaus, Amsterdam, The Netherlands; Dr. Dimitrious Roukos, Ioannina, Greece. The authors thank Dr. Cátia Moutinho, Porto, Portugal, for her excellent technical support to the panel of pathologists.

References

1. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: Cancer incidence, mortality and prevalence worldwide, Version 2.0. Lyon: IARC Press, 2004. IARC Cancer Base No. 5.
2. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—First American Cancer Society Award Lecture on cancer epidemiology and prevention. Cancer Res 1992;52:6735–40.
3. IARC monographs on the evaluation of the carcinogenic risks to humans. Schistosomes, liver flukes and *Helicobacter pylori*. Volume 61. Lyon: International Agency for Research on Cancer, 1994:177–241.
4. González CA, Sala N, Capellá G. Genetic susceptibility and gastric cancer risk. Int J Cancer 2002;100:249–60.
5. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;1: 1311–15.
6. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 1991;325:1127–31.
7. Sipponen P. Gastric cancer—a long-term consequence of *Helicobacter pylori* infection? Scand J Gastroenterol 1994;201 (Suppl):24–7.
8. Kuipers EJ, Uyterlinde AM, Pena AS, Roosendaal R, Pals G, Nelis GF, Festen HP, Meuwissen SG. Long-term sequelae of *Helicobacter pylori* gastritis. Lancet 1995;345:1525–8.
9. Helicobacter and Cancer Collaborative Group. Gastric cancer and *Helicobacter pylori*: a combined analysis of 12 case-control studies nested within prospective cohorts. Gut 2001;49:347–53.
10. Eslick GD, Lim LL, Byles JE, Xia HH, Talley NJ. Association of *Helicobacter pylori* infection with gastric carcinoma: a meta-analysis. Am J Gastroenterol 1999;94:2373–79.
11. Huang JQ, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. Gastroenterology 1998;114:1169–79.
12. Webb PM, Crabtree JE, Forman D, the EUROGAST Study Group. Gastric cancer, cytotoxin-associated gene A-positive *Helicobacter pylori*, and serum pepsinogens: an international study. Gastroenterology 1999;116:269–76.
13. Correa P, Schneider BG. Etiology of gastric cancer: what is new? Cancer Epidemiol Biomarkers Prev 2005;14:1865–68.
14. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 1995;55:2111–15.
15. Parsonnet J, Friedmann GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. Gut 1997;40:279–301.
16. Enroth H, Kraaz W, Engstrand L, Nyren O, Rohan T. *Helicobacter pylori* strain types and risk of gastric cancer: a case-control study. Cancer Epidemiol Biomarkers Prev 2000;9:981–4.
17. Ogura K, Maeda S, Nakao M, Watanabe T, Tada M, Kyutoku T, Yoshida H, Shiratori Y, Omata M. Virulence factors of *Helicobacter pylori* responsible for gastric diseases in Mongolian gerbil. J Exp Med 2000;192:1601–10.
18. Huang JQ, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. Gastroenterology 2003;125:1636–44.
19. Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S. Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. Science 2003;300:1430–34.
20. Stein M, Bagnoli F, Halenbeck R, Rappuoli R, Fantl WJ, Covacci A. c-Src/Lyn kinases activate *Helicobacter pylori* CagA through tyrosine phosphorylation of the EPIYA motifs. Mol Microbiol 2002;43:971–80.
21. Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 2002;295:683–6.
22. Masanori H. *Helicobacter pylori* CagA—a bacterial intruder conspiring gastric carcinogenesis. Int J Cancer 2006;119:1217–23.
23. Bagnoli F, Buti L, Tompkins L, Covacci A, Amieva MR. *Helicobacter pylori* CagA induces a transition from polarized to invasive phenotypes in MDCK cells. Proc Natl Acad Sci USA 2005;102: 16339–44.
24. Broutet N, Plebani M, Sakarovitch C, Sipponen P, Megraud F, Eurohepygast Study Group. Pepsinogen A, pepsinogen C, and gastrin as markers of atrophic chronic gastritis in European dyspeptics. Br J Cancer 2003;88:1239–47.
25. Knight T, Wyatt J, Wilson A, Greaves S, Newell D, Hengles K, Corlett M, Webb P, Forman D, Elder J. *Helicobacter pylori* gastritis and serum pepsinogen levels in a healthy population; development of a biomarker strategy for gastric atrophy in high-risk groups. Br J Cancer 1996;73:819–24.
26. Palli D, Decarli A, Cipriani F, Forman D, Amadori D, Avellini C, Giacosa A, Manca P, Russo A, Salkeld RM. Plasma pepsinogens, nutrients, and diet in areas of Italy at varying gastric cancer risk. Cancer Epidemiol Biomarkers Prev 1991;1:45–50.
27. Aromaa A, Kosunen TU, Knekt P, Maatela J, Teppo L, Heinonen OP, Harkonen M, Hakama MK. Circulating anti-*Helicobacter pylori* immunoglobulin A antibodies and low serum pepsinogen I level are associated with increased risk of gastric cancer. Am J Epidemiol 1996;144:142–9.
28. Varis K, Sipponen P, Laxen F, Samloff IM, Huttunen JK, Taylor PR, Heinonen OP, Albanes D, Sande N, Virtamo J, Harkonen M, Helsinki Gastritis Study Group. Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia. Scand J Gastroenterol 2000;35:950–6.
29. Riboli E, Hunt KJ, Slimani N, Ferrari P, Norat T, Fahey M, Charrondiere UR, Hemon B, Casagrande C, Vignat J, Overvad K, Tjonneland A et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;5:1113–24.
30. Ferrari P, Kaaks R, Fahey MT, Slimani N, Day NE, Pera G, Boshuizen HC, Roddam A, Boeing H, Nagel G, Thiebaut A, Orfanos P et al.

European Prospective Investigation into Cancer and Nutrition Study. Within- and between-cohort variation in measured macronutrient intakes, taking account of measurement errors, in the European Prospective Investigation into Cancer and Nutrition Study. Am J Epidemiol 2004;160:814–22.
31. Slimani N, Kaaks R, Ferrari P, Casagrande C, Clavel-Chapelon F, Lotze G, Kroke A, Trichopoulos D, Trichopoulou A, Lauria C, Bellegotti M, Ocke MC et al. European Prospective Investigation into Cancer and Nutrition (EPIC) calibration study: rationale, design and population characteristics. Public Health Nutr 2002;5:1125–45.
32. Ghiara P, Rossi M, Marchetti M, Di Tommaso A, Vindigni C, Ciampolini F, Covacci A, Telford JL, De Magistris MT, Pizza M, Rappuoli R, Del Giudice G. Therapeutic intragastric vaccination against *Helicobacter pylori* in mice eradicates an otherwise chronic infection and confers protection against reinfection. Infect Immun 1997;65:4996–5002.
33. Gonzalez CA, Pera G, Agudo A, Bueno-de-Mesquita HB, Ceroti M, Boeing H, Schulz M, Del Giudice G, Plebani M, Carneiro F, Berrino F, Sacerdote C et al. Fruit and vegetable intake and the risk of stomach and oesophagus adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). Int J Cancer 2006;118:2559–66.
34. González CA, Jakszyn P, Pera G, Agudo A, Palli D, Boeing H, Del Giudice G, Plebani M, Carneiro F, Nesi G, Berrino F, Vineis P et al. Meat and processed meat intake and the risk of stomach and oesophagus adenocarcinoma in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). J Natl Cancer Inst 2006;98: 345–54.
35. Ye W, Nyren O. Risk of cancers of the oesophagus and stomach by histology or subsite in patients hospitalised for pernicious anaemia. Gut 2003;52:938–41.
36. Ye W, Held M, Lagergren J, Engstrand L, Blot WJ, McLaughlin JK, Nyren O. *Helicobacter pylori* infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. J Natl Cancer Inst 2004;96:388–96.
37. Nomura AMY, Kolonel LN, Miki LK, Stemmermann GN, Wilkens LR, Goodman MT, Perez-Perez GI, Blaser MJ. *Helicobacter pylori*, pepsinogen, and gastric adenocarcinoma in Hawaii. J Infect Dis 2005;191:2075–81.
38. Wu AH, Crabtree JE, Bernstein L, Hawtin P, Cockburn M, Tseng CC, Forman D. Role of *Helicobacter pylori* CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. Int J Cancer 2003;103:815–21.
39. Hansen S, Melby KK, Aase S, Jellum E, Vollset SE. *Helicobacter pylori* infection and risk of cardia cancer and non-cardia gastric cancer: a nested case-control study. Scand J Gastroenterol 1999;34:353–60.
40. Limburg P, Qiao Y, Mark S, Wang G, Perez-Perez G, Blaser M, Wu Y, Zou X, Dong Z, Taylor P, Dawsey S. *Helicobacter pylori* seropositivity and subsite-specific gastric cancer risks in Linxian, China. J Natl Cancer Inst 2001;93:226–33.
41. Knekt P, Teppo L, Aromaa A, Rissanen H, Kosunen TU. *Helicobacter pylori* IgA and IgG antibodies, serum pepsinogen I and the risk of gastric cancer: changes in the risk with extended follow-up period. Int J Cancer 2006;119:702–5.
42. Malfertheiner P, Peitz U. The interplay between *Helicobacter pylori*, gastro-oesophageal reflux disease, and intestinal metaplasia. Gut 2005;54 (Suppl I):i13–i20.