Gastric Parietal Cell Antibodies, Helicobacter Pylori Infection, and Chronic Atrophic Gastritis: Evidence from a Large Population-based Study in Germany

Yan Zhang1, Melanie N. Weck1, Ben Schöttker1, Dietrich Rothenbacher1,2, and Hermann Brenner1

Abstract

Background: Striking similarities between autoimmune gastritis and Helicobacter Pylori (H. pylori)-associated gastritis have suggested a potential link between these two pathologic conditions in the progression of chronic atrophic gastritis (CAG); however, evidence has remained conflicting.

Methods: Serum pepsinogen I and II, and antibodies against H. pylori in general, the cytotoxin-associated gene A protein (CagA) and parietal cells were measured by ELISA in 9,684 subjects aged 50 to 74 years. Antigastric parietal cell antibody (APCA) prevalence was examined in the overall population and according to sex, age, and H. pylori serostatus. The association between APCA prevalence and CAG was assessed by logistic regression, overall and according to H. pylori status, controlling for potential confounding factors.

Results: Overall APCA prevalence was 19.5%. APCA prevalence was strongly associated with CAG, and the association was increasing with increasing severity of CAG. Furthermore, the association between APCA and CAG was even stronger among H. pylori-negative subjects [odds ratio (OR) = 11.3; 95% confidence interval (CI): 7.5–17.1] than among H. pylori-positive subjects (OR = 2.6; 95% CI: 2.1–3.3).

Conclusions: APCA may play a role on the development of gastric atrophy, irrespective of H. pylori infection.

Impact: Assessment of APCA might be a useful complement to established markers (such as pepsinogens and H. pylori antibodies) in screening for CAG. Cancer Epidemiol Biomarkers Prev; 22(5); 821–6. ©2013 AACR.

Introduction

Chronic atrophic gastritis (CAG) is a well-known precursor for gastric cancer (1, 2). Autoimmune gastritis is a special form of CAG, characterized by the presence of circulating autoantibodies against H+/K+-adenosine triphosphatase (H+/K+-ATPase), the gastric proton pump located on parietal cells (antiparietal cell antibody, APCA; refs. 3, 4). Following the discovery of Helicobacter pylori (H. pylori) and the establishment of a causal relationship between H. pylori infection and CAG (1, 2), several studies have reported that a considerable number of patients with H. pylori infection also expressed autoantibodies against H+/K+-ATPase (5–8). The presence of these autoantibodies in H. pylori infection was also significantly associated with higher fasting serum gastrin levels, a lower pepsinogen I to II ratio, and reduced secretion of gastric acid (8–10). In addition, pernicious anemia, which was earlier considered as an exclusive complication of autoimmune gastritis (11), was also shown to be accompanied by H. pylori infection (12–14). On the basis of these histologic and clinical similarities, the hypothesis has been raised that classic autoimmune gastritis may be triggered by H. pylori infection (15, 16). However, pertinent evidence reported to date was primarily based on relatively small studies from the clinical setting. As a substantial proportion of CAGs are asymptomatic (17), and autoimmune gastritis is often an incidental diagnosis (18), large population-based studies are needed for a more comprehensive epidemiologic assessment. We therefore assessed the association of CAG with APCA positivity in a large population-based study, paying particular attention to the possible role of H. pylori infection.

Materials and Methods

Study population

The analyses are based on the baseline data of the ESTHER study (Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung), a large population-based cohort study conducted among older adults in Saarland, Germany. Details of the study design have been reported elsewhere (19, 20). Briefly, 9,949 participants ages 50 to 74 years (mean age, 62 years) were recruited by their general practitioner during a general health check-up between July 2000 and December

Authors’ Affiliations: 1Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Im Neuenheimer Feld, Heidelberg; and 2Institute of Epidemiology and Medical Biometry, University of Heidelberg, Im Neuenheimer Feld, Heidelberg, Germany. Phone: 49-6221-421300; Fax: 49-6221-421302; E-mail: h.brenner@dkfz.de

doi: 10.1158/1055-9965.EPI-12-1343
©2013 American Association for Cancer Research.
2002. Written informed consent was obtained from all participants. The study was approved by the ethics committees of the medical faculty of the University of Heidelberg and of the medical board of the State of Saarland, Germany.

**Data collection**

*Questionnaire.* A standardized self-administrated questionnaire was completed by each participant, collecting information on sociodemographic characteristics, lifestyle factors, medical history, health status, and family history of major diseases.

*Laboratory analyses.* Serum samples were obtained from participants at the day of the health check-up, mailed to a central laboratory, and stored at \(-80^\circ\)C until laboratory analyses. Serum concentrations of pepsinogen I and II were measured by ELISA (Biohit). Immunoglobulin G (IgG) antibodies against *H. pylori* in general and the cytotoxin-associated gene A protein (CagA) were determined by ELISAs (Helicobacter pylori ELISA (Screen) and Helicobacter pylori p120 (CagA) ELISA, ravo Diagnostika). Classification of infection status was made according to the manufacturer’s instructions, and borderline results were treated as negative. The sensitivity and specificity of the *H. pylori* serologic test was 96% and 74%, respectively, when compared with results of the \(^{13}\)C-urea breath test in a previous validation study conducted in 205 adult blood donors aged 40 to 68 years (21). APCAs against H+/K+-ATPase antigen were analyzed by ELISA (Euroimmun). APCA concentration was measured as optical density by plate-reader Anthos Reader 2010 (Mikrosysteme GmbH) and calculated according to point-to-point calibration curve. Serum dilution of 1:10 (serum:buffer) was used for initial measurement. Samples with values above the highest calibrator were diluted further until their value was within the range of calibration curve. The dilution factor was then applied to calculate APCA concentration. Test results were interpreted according to the manufacturer’s instructions. All serologic examinations were carried out in a blinded fashion in the same laboratory.

**Statistical analysis**

Of the 9,949 participants recruited in the ESTHER study, 7 with a previous diagnosis of gastric cancer and 258 without APCA concentrations (due to technical reasons unrelated to characteristics of the study population) were excluded from statistical analyses. The remaining 9,684 participants were characterized with respect to sociodemographic characteristics, lifestyle factors, family and own medical history, *H. pylori* infection, and CAG. The presence of CAG cases was determined by the following serology-based definition which has been commonly used in previous studies (21): pepsinogen I <70 ng/mL and pepsinogen I/II <3.

The prevalence of APCA was examined according to age, sex, and *H. pylori* serostatus. Then, ORs and corresponding 95% confidence intervals (CI) for the association of APCA prevalence with CAG (overall and stratified by *H. pylori* status, the latter further stratified according to CagA status) were estimated by logistic regression models, additionally controlling for age (years), sex, educational level (low/intermediate/high), smoking status (former smoker/current smoker/never smoker), alcohol consumption (g/week), and family history of gastric cancer (yes/no). A sensitivity analysis was conducted by excluding subjects who reported having had a history of *H. pylori* infection (n = 377), duodenal ulcer (n = 929), or both (n = 270), as these participants would most likely have had *H. pylori* eradication therapy. Finally, we assessed the association of APCA positivity with various levels of severity of CAG which were defined according to quintiles of pepsinogen I level (20), with the lowest quintile indicating the most severe form of CAG. Again, these analyses were stratified according to *H. pylori* infection. Moreover, the analyses were repeated after excluding subjects who were *H. pylori* negative but CagA positive, as this constellation might be an indicator of past infection with *H. pylori* (22).

All analyses were conducted using the statistical software package SAS9.2 (SAS Institute), and 2-sided P values of less than 0.05 were considered statistically significant.

**Results**

Table 1 shows the characteristics of the study population. It included more women (54.6%) than men and the mean age was 62 years. Three quarters of the study population had less than 10 years of school education, and half of the participants had ever smoked. Approximately 6% of the subjects had a family history of gastric cancer. More than half of the participants presented serologic evidence of current or previous *H. pylori* infection (seropositive for either *H. pylori* in general or CagA antibodies). The serology-based prevalence of CAG in the study population was 5.4%.

Overall, approximately 20% of study participants have shown elevated levels of APCAs, with little variation by sex (P = 0.19; Table 2). Overall, APCA seroprevalence slightly increased with age (P \(_{\text{trend}}\) = 0.002), a trend that was though observed in men only (P \(_{\text{trend}}\) = 0.0003). APCA was slightly more prevalent among *H. pylori* infected subjects than among those without *H. pylori* infection (20.7% versus 18.4%, P = 0.006), and this difference in prevalence was due to a higher prevalence in participants with CagA-negative infection. Furthermore, the increase of APCA prevalence with age was confined to *H. pylori* infection (20.7% versus 18.4%, P = 0.006), but not observed among *H. pylori*-positive subjects.

Table 3 shows the associations between APCA prevalence and CAG derived from logistic regression. Overall, APCA prevalence was associated with an almost 4-fold increased risk of CAG (46.2% versus 18.0%, adjusted OR = 3.8; 95% CI: 3.1–4.7). Controlling for potential confounders as well as inclusion of *H. pylori* infection in the multiple regression model both had very little impact on the estimated association. However, a significant interaction between APCA status and *H. pylori* infection was detected...
APCA prevalence were 19.2%, 20.2%, and 18.4% among the overall, *H. pylori*-positive and *H. pylori*-negative subjects, respectively, and ORs for the association between APCA prevalence and CAG were 3.9 (95% CI, 3.2–4.9), 2.8 (95% CI, 2.2–3.6), and 10.5 (95% CI, 6.7–16.7), respectively.

Table 4 shows the association of APCA positivity with various levels of severity of CAG. In the absence of *H. pylori* infection, the association with APCA strongly increased with increasing severity of CAG defined by PGI levels. This pattern persisted even after excluding subjects who were *H. pylori* negative and CagA positive (data not shown). A much less pronounced dose-response pattern was seen among *H. pylori*-positive subjects.

### Discussion

In this large population-based study among older adults, we found APCA seropositivity to be strongly associated with CAG. While the association increased with the increasing level of disease severity in both *H. pylori*-negative and *H. pylori*-positive participants, the strength of the association and the dose-response pattern were much more pronounced in the former than in the latter group. Taken together, these results suggest that APCA may play a major role in the progression of CAG, independent from *H. pylori* infection.

Autoimmune gastritis is characterized by a chronic inflammatory infiltration in the gastric corpus mucosa as a result of autoimmune destruction mediated by APCAs (16), which is silent initially and becomes clinically manifest by the development of pernicious anemia (11) or is incidentally diagnosed due to other disorders (18). Most previous studies investigating the relation of CAG with autoimmune response and *H. pylori* infection were based on clinically selected populations and may therefore not have included the majority of asymptomatic CAG cases. To our knowledge, this is by far the largest population-based study that has systematically investigated the etiology of CAG, paying attention to the role of APCA and *H. pylori* infection simultaneously. In opposition to the previous finding that APCA prevalence increased with age in *H. pylori*-infected subjects (23), the age-dependence of APCA prevalence was confined to subjects without *H. pylori* infection in our study. A study conducted among patients with gastric diseases reported that the correlation between APCA levels and the extent of gastric atrophy (measured by pepsinogen I/II ratio) was limited to the *H. pylori*-positive group (24). In our large population-based study, the association of APCA and CAG increased more strongly with atrophic severity in *H. pylori*-negative than *H. pylori*-positive subjects.

Previous studies have reported on several sporadic CAG cases who were APCA positive but without any evidence of *H. pylori* infection. For example, APCA appeared during 32 years of follow-up in a subject who was *H. pylori* negative at both baseline and final examination (25), and studies conducted in childhood populations failed to detect *H. pylori* infection for some children.
with APCA (15, 26, 27). In addition, APCA and *H. pylori* infection have been suggested to be associated with different HLA-DQ haplotypes in patients with type I diabetes (28). In another study, APCA antibodies did not decrease after cure of *H. pylori* infection over 1 year for a portion of patients (29). In line with our results, these findings suggest that APCA-mediated autoimmune gastritis may frequently be induced by other unknown factors or diseases that are unrelated to *H. pylori* infection.

Of note, it has been suggested that histologically defined early stages of autoimmune gastritis can be successfully treated by *H. pylori* eradication (30–32), which supported the hypothesis that infection with *H. pylori* infection may trigger APCA-mediated autoimmune response in a subset of infected patients. However, conflicting results have also been reported (24, 29). Simultaneous occurrence of *H. pylori* infection and autoimmune reaction observed in previous investigations (5–8) or our study population cannot be interpreted to support a causal relationship between bacterial infection and autoimmune response. Because of the cross-sectional study design with simultaneous ascertainment of infection, autoimmune response and CAG, temporal relationships between occurrence of bacterial infection, autoimmune response and development of CAG as well as potential disease-related loss of *H. pylori* infection could not be observed. Longitudinal studies with repeated measurement of these parameters during life course would be needed for definitive clarification of the interdependencies.

*Helicobacter pylori* strains that express the *cagA* gene located at the end of the *cag* pathogenicity island have been identified to cause enhanced inflammatory response, elevated serum gastrin levels, and increased risk for gastric malignancies (33). Only 2 previous studies have examined the role of this virulence factor in relation to APCA. One study reported that the presence of APCA was not associated with CagA status based on 49 patients (34), whereas in the other study, APCA was detected in 10 of 19 (52.6%) CagA-positive and 19 of 23 (82.6%) CagA-negative subjects (*P* < 0.05) (35). Our large study also found that APCA was more prevalent in CagA-negative strains.

### Table 2. Antiparietal cell antibodies seroprevalence in the study population

<table>
<thead>
<tr>
<th>Group</th>
<th>Overall N (%)</th>
<th>Females N (%)</th>
<th>Males N (%)</th>
<th>H. pylori + N (%)</th>
<th>H. pylori − N (%)</th>
<th>H. pylori + CagA + N (%)</th>
<th>H. pylori + CagA − N (%)</th>
<th>H. pylori − CagA + N (%)</th>
<th>H. pylori − CagA − N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1,889 (19.5)</td>
<td>1,007 (19.0)</td>
<td>882 (20.1)</td>
<td>1,016 (20.7)</td>
<td>864 (18.4)</td>
<td>486 (18.9)</td>
<td>527 (22.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50–54 years</td>
<td>279 (17.0)</td>
<td>163 (17.1)</td>
<td>116 (16.2)</td>
<td>137 (18.9)</td>
<td>141 (15.6)</td>
<td>70 (18.6)</td>
<td>67 (19.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>55–59 years</td>
<td>302 (18.4)</td>
<td>170 (18.8)</td>
<td>132 (17.8)</td>
<td>149 (20.6)</td>
<td>153 (16.8)</td>
<td>72 (19.7)</td>
<td>77 (21.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60–64 years</td>
<td>549 (20.8)</td>
<td>288 (20.2)</td>
<td>261 (21.4)</td>
<td>291 (21.4)</td>
<td>253 (20.0)</td>
<td>143 (20.2)</td>
<td>147 (22.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65–69 years</td>
<td>433 (19.4)</td>
<td>222 (18.4)</td>
<td>211 (20.7)</td>
<td>253 (20.8)</td>
<td>177 (17.8)</td>
<td>117 (17.9)</td>
<td>135 (24.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70–74 years</td>
<td>326 (21.4)</td>
<td>164 (19.6)</td>
<td>162 (23.5)</td>
<td>186 (20.7)</td>
<td>140 (22.5)</td>
<td>84 (18.1)</td>
<td>101 (23.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P&lt;sub&gt;rend&lt;/sub&gt;</td>
<td>0.002</td>
<td>0.56</td>
<td>0.0003</td>
<td>0.45</td>
<td>0.002</td>
<td>0.56</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Association between APCA seroprevalence and CAG according to *H. pylori* serostatus

<table>
<thead>
<tr>
<th>Group</th>
<th>APCha serostatus</th>
<th>N</th>
<th>CAG</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Model 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Model 2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall</td>
<td>negative</td>
<td>7,490</td>
<td>271</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>1,820</td>
<td>233</td>
<td>12.8</td>
</tr>
<tr>
<td>H. pylori−</td>
<td>negative</td>
<td>3,709</td>
<td>34</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>834</td>
<td>86</td>
<td>10.3</td>
</tr>
<tr>
<td>H. pylori+</td>
<td>negative</td>
<td>3,781</td>
<td>237</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>986</td>
<td>147</td>
<td>14.9</td>
</tr>
<tr>
<td>H. pylori+/CagA−</td>
<td>negative</td>
<td>1,755</td>
<td>46</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>508</td>
<td>66</td>
<td>13.0</td>
</tr>
<tr>
<td>H. pylori+/CagA+</td>
<td>negative</td>
<td>2,017</td>
<td>189</td>
<td>9.4</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>474</td>
<td>81</td>
<td>17.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>OR without adjustment.

<sup>b</sup>OR adjusted for age, sex, family history of gastric cancer, educational level, smoking status, alcohol consumption.

<sup>c</sup>OR adjusted for *H. pylori* infection.
negative infection, and the association between APCA prevalence and CAG was much stronger among subjects with CagA-negative infection compared with subjects with CagA-positive infection. This observation gives further support to the suggestion that APCA presence is not primarily triggered by *H. pylori* virulence, and that a substantial proportion of *H. pylori* infections, in particular CagA positive infections, are likely to lead to CAG through pathways other than autoimmune response. On the other hand, another member of the *cag* pathogenicity island, CagE, has been shown to induce parietal cell apoptosis *in vitro* (36). Additional virulence factors of this infectious agent relevant to induction of autoimmune response warrant further investigation.

In the interpretation of our study, additional limitations have to be considered. CAG was not confirmed by histology, as it is impractical to implement gastroscopy in a large-scale epidemiologic study outside special-risk groups. Although gastroscopy-based histologic diagnosis is often considered as the gold standard for determining atrophic gastritis, it also suffers from specific limitations, such as sampling errors (37), and intra- and interobserver variation (38). Advantages of measurements of serum pepsinogen levels are the high degree of standardization and the fact that they reflect an integrative quantitative measure of the gastric mucosal function. Serologic measurement of *H. pylori* infection is likewise less than perfect and may have led to some alteration of differences between infected and non-infected participants by misclassification of infection status.

In summary, despite its limitations, our large population-based study substantially expands existing evidence on the interrelationship between *H. pylori* infection and APCA-mediated autoimmune response in the development of CAG. Our results suggest that *H. pylori* infection and APCA-mediated autoimmune response might, for the most part, be independent, distinct pathways, rather than causally related pathways leading to CAG. Assessment of APCA might be a useful complement to established markers (such as pepsinogens and *H. pylori* antibodies) in screening for CAG.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

Conception and design: M. Weck, D. Rothenbacher, H. Brenner

Development of methodology: M. Weck

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Weck, D. Rothenbacher, H. Brenner

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y. Zhang, H. Brenner

Writing, review, and/or revision of the manuscript: Y. Zhang, B. Schöttler, D. Rothenbacher, H. Brenner

Study supervision: H. Brenner

### Grant Support

This study was supported in part by grants from the Baden Württemberg Ministry of Science, Research and Arts, Germany.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 3, 2012; revised February 13, 2013; accepted February 21, 2013; published OnlineFirst March 1, 2013.

---

**References**


Gastric Parietal Cell Antibodies, *Helicobacter Pylori* Infection, and Chronic Atrophic Gastritis: Evidence from a Large Population-based Study in Germany

Yan Zhang, Melanie N. Weck, Ben Schöttker, et al.


Updated version  Access the most recent version of this article at: doi:10.1158/1055-9965.EPI-12-1343

Cited articles  This article cites 38 articles, 5 of which you can access for free at: http://cebp.aacrjournals.org/content/22/5/821.full.html#ref-list-1

Citing articles  This article has been cited by 3 HighWire-hosted articles. Access the articles at: /content/22/5/821.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.