Sibship size, *Helicobacter pylori* infection and chronic atrophic gastritis: a population-based study among 9444 older adults from Germany

Lei Gao,1 Melanie N Weck,1 Elke Raum,1 Christa Stegmaier,2 Dietrich Rothenbacher1 and Hermann Brenner1*

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**Background** Early-life social environment has been suggested to play an important role during the development of *Helicobacter pylori*-related gastric diseases. We aimed to assess the association of sibship size with *H. pylori* infection and chronic atrophic gastritis (CAG) in a population-based study from Germany.

**Methods** In the baseline examination of ESTHER, a study conducted in Saarland, serological measurements of pepsinogen I and II and *H. pylori* antibodies were taken in 9444 participants aged 50–74 years. Information on potential risk factors and medical history were obtained by self-administered standardized questionnaire.

**Results** A strong dose–response relationship between sibship size and *H. pylori* seroprevalence was observed ($P < 0.01$). Adjusted odds ratios (ORs) 95% confidence interval (CI) for *H. pylori* seropositivity for subjects with 4, 5, 6 and 7 or more siblings compared with subjects without siblings were 1.45 (1.20–1.77), 1.83 (1.50–2.22) and 1.84 (1.47–2.31), respectively. A large sibship size was also associated with an increased risk of CAG with an adjusted OR of 1.42 (1.01–2.01) for 7 or more compared with less than or equal to 2 siblings. This association was attenuated but not entirely eliminated after additional adjustment for *H. pylori* infection. Notably, a significant association between large sibship size and CAG was also found among *H. pylori*-negative subjects.

**Conclusions** Our results suggest that large sibship size is associated with increased *H. pylori* prevalence and CAG risk. The association with CAG risk may be mediated at least in part by *H. pylori* infection. However, mechanisms other than *H. pylori* infection may contribute to the ‘sibling effect’ as well.

**Keywords** Sibship size, chronic atrophic gastritis, *Helicobacter pylori*

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**Introduction**

Chronic atrophic gastritis (CAG) is an established precursor of gastric cancer, which is the second most common cause of cancer-related death worldwide.1,2 *Helicobacter pylori* infection is known as a key cause of triggering gastric inflammation and carcinogenesis.3 Acquisition of *H. pylori* infection is thought to occur
H. pylori might be mediated through increased risk of infection among those with more siblings.\textsuperscript{3–8} However, previous findings from studies with relatively small numbers of gastric cancer patients need to be verified in larger populations and with respect to other H. pylori-related diseases. Furthermore, evidence regarding the ‘sibling effect’ on the development of CAG is sparse.\textsuperscript{9} The objective of this study was to assess the association of sibship size with H. pylori infection and CAG risk in a large population-based study among older adults from Germany, thereby providing further insights into ‘sibling effect’ during gastric carcinogenesis.

**Materials and methods**

**Study population**

Our analyses are based on baseline data of ESTHER (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 Germany to investigate new avenues of prevention and early detection of chronic diseases in the elderly. Details of the study design have been described elsewhere.\textsuperscript{10} Briefly, 9953 participants (45\% males), aged 50–74 years (mean age: 62 years), were recruited between July 2000 and December 2002 by their general practitioners during a general health check-up in Saarland, a state in the south-west of Germany. 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. Written informed consent was obtained from each participant.

**Data collection**

**Questionnaires**

A standardized questionnaire was completed by every participant, providing information on socio-demographic characteristics, medical history, health status, family history and lifestyle factors. Current alcohol consumption was quantified by the self-reported average alcohol consumption within the past 12 months in consumption volumes typical for southern Germany. Smoking status was classified into three groups (current smoker, former smoker and never smoker).

**Serological examinations**

Serum samples were obtained from all participants and stored at \(-80^\circ\text{C}\). Serum concentrations of pepsinogen (PG) I and II were measured by ELISA (Biohit, Helsinki, Finland). Presence of CAG was determined by the following serological definition, which has been used in previous epidemiological studies: PG I < 70 ng/ml and PG I/PG II < 3.\textsuperscript{11} Samples were additionally analysed by ELISA for the presence of immunoglobulin G (IgG) antibodies both against H. pylori in general and specific to the CagA protein of H. pylori (H. pylori Screening ELISA and H. pylori p120 (CagA) ELISA, ravo Diagnostika, Freiburg, Germany). Classification of serostatus followed the manufacturer’s instructions; borderline results were treated as negative. To minimize potential underestimation of the association between sibship size and H. pylori prevalence due to clearance of the infection in elderly people or people with gastric lesions,\textsuperscript{12,13} H. pylori infection was defined by positivity in either the H. pylori or the CagA assay.\textsuperscript{14} All analyses were carried out in a blind fashion in the same laboratory.

**Statistical analysis**

The associations of sibship size with H. pylori prevalence and CAG risk were estimated by means of odds ratios (ORs) and 95\% confidence intervals (CIs) using unconditional multiple logistic regression. Sibship size was categorized into 0, 1, 2, 3, 4, 5, 6, 7 or more siblings and 0–2, 3–6, 7 or more siblings for the former and for the latter (much less common) outcomes, respectively. The association estimates were adjusted for age and sex, and, in addition, for other potential risk factors (education, family history of gastric cancer, smoking and alcohol drinking). The analysis of CAG risk was repeated after additional adjustment for H. pylori infection and additional analyses were carried out separately for H. pylori-positive and H. pylori-negative subjects. Evidence for interaction between sibship size and H. pylori infection was assessed using the likelihood ratio test by comparing logistic regression models with and without an interaction term. Tests for trend by sibship size were performed by treating sibship size as continuous variable in the logistic regression analysis.

In additional sensitivity analyses, the association between sibship size and CAG risk was evaluated after excluding 70–74-year-old people or those with most severe forms CAG (lowest 20\% of PG I serum level, PG I < 8.87 ng/ml),\textsuperscript{13} to minimize potential bias caused by the clearance of H. pylori infection and subsequent disappearance of H. pylori antibodies in elderly people or people with severe gastric lesions.\textsuperscript{12,13} The analyses were also repeated using the definition of H. pylori serostatus based on H. pylori assay only (i.e. leaving CagA serostatus out of account). All statistical analyses were carried out using SAS statistical software, release 9.1.

**Results**

Of the 9953 participants in the ESTHER baseline examination, 7 were excluded from the analyses
because of a history of gastric cancer and PG concentrations were not available, leading to a final study population of 9444. Table 1 shows the main characteristics of the study population as well as prevalence of Helicobacter pylori infection, CAG disease status and sibship size. Overall, 4244 men and 5200 women were included with a mean age of 62 years. The prevalence of CAG and Helicobacter pylori seropositivity was 5.7 and 52.6%, respectively. Prevalence of Helicobacter pylori infection increased with age from 45.6% in the age group of 50–54 years to 60.3% in the age group of 70–74 years (P for trend < 0.05), but no substantial difference was observed between men (52.9%) and women (52.4%). About half of the participants had 1 or 2 siblings, but there were also a large number of participants with 0 or 3 or more siblings.

As Table 2 shows, there was a strong dose–response relationship between the number of siblings and Helicobacter pylori infection (P < 0.01). Adjusted ORs (95% CI) for subjects with 4, 5–6 and 7 or more siblings compared with subjects without siblings were 1.45 (1.20–1.77), 1.83 (1.50–2.22) and 1.84 (1.47–2.31), respectively, after controlling for age, sex, education, family history of gastric cancer, smoking and alcohol drinking.

Table 3 shows the association between sibship size and CAG risk. A large number of siblings (7 or more) was found to be associated with a significantly increased risk of CAG (OR = 1.42; 95% CI 1.01–2.01) compared with a smaller number of siblings (2 or less) after adjustment for age, sex, education, family history of gastric cancer, smoking and alcohol drinking in multivariate analysis. This association was attenuated and no longer statistically significant after additional adjustment for Helicobacter pylori infection may present part of the intermediate pathway between sibship size and CAG risk. However, a strong association was also observed among Helicobacter pylori-negative subjects with an adjusted OR of 2.12 (95% CI 1.09–4.14). A statistical interaction between sibship size and Helicobacter pylori infection was not significant though (P = 0.43).

In additional sensitivity analyses, excluding 1487 subjects who were 70–74 years old or 107 subjects with most severe forms of CAG (20% of total CAG patients), or using the definition of Helicobacter pylori infection based on Helicobacter pylori assay only (leaving CagA serostatus out of account) did not materially alter the results (data not shown).

**Discussion**

To our knowledge, this is the largest epidemiological study to evaluate the association of sibship size with Helicobacter pylori infection and CAG to date. Our results are consistent with the hypothesis that a large sibling number is positively related to CAG, and hence gastric carcinogenesis.

As an indirect indicator of living conditions in childhood, sibship size is found to be strongly related to
People who have more siblings often grow up in a more overcrowded accommodation, with greater exposures to early infections and less utilization of health care service. These factors may contribute to health in adulthood either through influencing living environment or through the establishment of behaviour patterns. As early as 1990, Drumm and colleagues suggested that the intrafamilial clustering of *H. pylori* infection may reflect person-to-person spread of these bacteria.

### Table 2

<table>
<thead>
<tr>
<th>Number of siblings</th>
<th>HP+ n/N (%)</th>
<th>Crude</th>
<th>Adjusteda</th>
<th>Adjustedb</th>
<th>Adjustedc</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–2</td>
<td>288/5480 (5.3)</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>3–6</td>
<td>172/2982 (5.8)</td>
<td>1.10 (0.91–1.34)</td>
<td>1.05 (0.87–1.27)</td>
<td>1.07 (0.88–1.31)</td>
<td>1.00 (0.82–1.23)</td>
</tr>
<tr>
<td>7 or more</td>
<td>47/560 (8.4)</td>
<td>1.65 (1.20–2.28)</td>
<td>1.53 (1.11–2.10)</td>
<td>1.42 (1.01–2.01)</td>
<td>1.27 (0.90–1.80)</td>
</tr>
<tr>
<td>P for trend d</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.06</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

HP+: includes people who were seropositive in either the *H. pylori* or the CagA assay.

aPartly adjusted for age and sex.

bFully adjusted for age, sex, education, family history of gastric cancer, smoking and alcohol drinking.

In trend tests, sibship size was treated as continuous variables in the logistic regression analysis.

Values in bold indicate *P* < 0.05.

### Table 3

The impact of number of siblings on CAG risk stratified by *H. pylori* serostatus

<table>
<thead>
<tr>
<th>Number of siblings</th>
<th>Cases n/N (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude</td>
</tr>
<tr>
<td>Total population</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–2</td>
<td>221/2675 (7.9)</td>
<td>Ref.</td>
</tr>
<tr>
<td>3–6</td>
<td>143/1703 (8.4)</td>
<td>1.07 (0.86–1.34)</td>
</tr>
<tr>
<td>7 or more</td>
<td>35/362 (9.7)</td>
<td>1.25 (0.86–1.82)</td>
</tr>
<tr>
<td>P for trend d</td>
<td>&lt;0.03</td>
<td>&lt;0.45</td>
</tr>
</tbody>
</table>

²Partly adjusted for age and sex.

³Fully adjusted for age, sex, education, family history of gastric cancer, smoking and alcohol drinking.

In trend tests, sibship size was treated as continuous variables in the logistic regression analysis.

Values in bold indicate *P* < 0.05.
Teh et al.\textsuperscript{19} found a dose–response relationship between sibship size and \textit{H. pylori} seropositivity in a Taiwanese population in 1994. This association was also observed in a number of different populations in further studies that suggested that \textit{H. pylori} infection is mainly acquired through person-to-person transmission during early childhood.\textsuperscript{20–26} However, the association of sibship size or presence of \textit{H. pylori}-infected siblings does not necessarily imply siblings to be the main source of \textit{H. pylori} infection in early childhood. There is increasing evidence that the infection is primarily transmitted from mothers to their children, at least in developed countries.\textsuperscript{27} In case of multiple children per family this may also account to a large extent for apparent clustering of the infection among children, besides possible transmission between children.\textsuperscript{28}

As \textit{H. pylori} infection is a key cause of triggering chronic gastric inflammation that progresses to atrophy, metaplasia, dysplasia and gastric cancer,\textsuperscript{29} the effect of sibship size on gastric carcinogenesis was investigated in several studies.\textsuperscript{5–7} Positive associations were observed between the number of siblings and the risk of developing gastric cancer.\textsuperscript{5–7} To our knowledge, however, the ‘sibling effect’ has not been widely addressed with respect to the development of CAG, which is an established precursor of gastric cancer. Kikuchi \textit{et al.}\textsuperscript{9} observed a positive relationship between sibship size and gastric atrophy among \textit{H. pylori}-infected subjects from Japan.

In the present study, a clear dose–response relation between sibship size and \textit{H. pylori} infection was observed. Furthermore, we found a significant positive association between large sibship size and the risk of developing CAG. Our results therefore support the hypothesis that the ‘sibling effect’ plays an important role during the process of gastric carcinogenesis. However, \textit{H. pylori} infection seemed to explain only a part, but not all of the observed sibship–CAG relationship. The fact that the association between sibship size and CAG was also pronounced among \textit{H. pylori} seronegative subjects suggests that factors other than \textit{H. pylori} infection contribute to the ‘sibling effect’ as well. The number of siblings is likely to be associated with multiple environmental, behavioural and biological factors, and it is well conceivable that some of them may be also associated with the development of CAG and gastric cancer. For example, family size in childhood may affect family economic status and dietary behaviour, such as salted food and vegetable intake, which have been linked to the risk of gastric cancer.\textsuperscript{10,31} Whether there are genetic factors that predispose people with large sibship size to develop gastric cancer needs further investigation. Such studies may be helpful to fully understand the process of gastric carcinogenesis.

In the interpretation of our data, some limitations have to be considered. First, clearance of \textit{H. pylori} colonization in the stomach of subjects with severe forms CAG and subsequent loss of antibodies against \textit{H. pylori} may often conceal past infection in serological studies.\textsuperscript{12,32} Secondly, data of socio-demographic and lifestyle factors were based on a self-administered standardized questionnaire. Potential bias caused by inaccurate response cannot, therefore be, excluded. In addition, because the study participants were recruited during a voluntary health check-up, the study population is not a random sample. However, participants are similar to the total population of the State of Saarland with respect to major socio-demographic and risk factors.\textsuperscript{33} Thirdly, the definition of CAG based on serum PG concentrations cannot be claimed as being perfect. However, high levels of agreement with classification by gastroscopy with subsequent histological examination of biopsies have been observed,\textsuperscript{34} even though the latter has been shown to bear considerable observer variation\textsuperscript{35} and to suffer from sampling error.\textsuperscript{36} An advantage of PG-based definition of CAG is that PG measurements are highly standardized and suitable for application in large-scale population-based studies.

In conclusion, our results are consistent with the hypothesis that large sibship size is associated with increased risks of \textit{H. pylori} infection in childhood and the development of related gastric disease in later life. However, mechanisms other than \textit{H. pylori} infection may contribute to the sibship–CAG association as well. Further studies are needed to fully understand the underlying mechanisms of the ‘sibling effect’ during the process of gastric carcinogenesis.

**Acknowledgements**

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**Conflict of interest:** None declared.

**KEY MESSAGE**

- Results based on this larger population-based study suggest that large sibship size is associated with increased \textit{H. pylori} prevalence and CAG risk. The association with CAG risk may be mediated at least in part by \textit{H. pylori} infection. This finding supports the positive relationship between sibship size and gastric cancer observed in previous study.
References