SHORT COMMUNICATION

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Associations of *Helicobacter pylori* infection and chronic atrophic gastritis with accelerated epigenetic ageing in older adults

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Background: *Helicobacter pylori* (HP) infection and chronic atrophic gastritis (CAG) have shown strong associations with the development of gastric cancer. This study aimed to examine whether both risk factors are associated with accelerated epigenetic ageing, as determined by the 'DNA methylation age', in a population-based study of older adults ($n = 1477$).

Methods: Serological measurements of HP antibodies and pepsinogen I and II for CAG definition were obtained by ELISA kits. Whole blood DNA methylation profiles were measured by Illumina Human Methylation450K Beadchip. DNA methylation ages were calculated by two algorithms proposed by Horvath and Hannum *et al*.

Results: After adjusting for potential covariates in linear regression models, we found that HP infection, infection with virulent HP strains (CagA+) and severe CAG were significantly associated with an increase in DNA methylation age by ~0.4, 0.6 and 1 year (all *P*-values <0.05), respectively.

Conclusions: Our study indicates that both CagA+ HP infection and CAG go along with accelerated epigenetic ageing.

Gastric cancer is the third leading cause of cancer mortality globally (Ferlay *et al*, 2015). *Helicobacter pylori* (HP) is related aetiologically to gastric cancer by playing a key role in the development of gastric carcinogenesis (Graham, 2015). HP infection also shows a causal relationship with chronic atrophic gastritis (CAG), a chronic, strongly age-related condition, which is a critical precursor lesion of gastric cancer (Weck and Brenner, 2008) and related to other chronic, age-related health outcomes, such as vitamin B_{12} deficiency and cardiovascular disease (CVD) (Lewerin *et al*, 2008; Franceschi *et al*, 2009). Previous studies have shown that HP infection is closely related to DNA methylation changes in gastric mucosa and whole blood samples (Maekita *et al*, 2006; Zhang *et al*, 2016). One of the conceivable causes is that the oxidative stress induced by HP infection contributes to the accelerated senescence of cells and ageing (Naito and Yoshikawa, 2002; Pandey and Rizvi, 2010). Moreover, based on the age-related alterations of DNA methylation, researchers have established the concept of DNA methylation age (Hannum *et al*, 2013; Horvath, 2013), and have employed the discrepancy between DNA methylation age and chronological age (termed 'age acceleration'(AA)) as an informative biomarker of ageing and ageing-related health outcomes. Given the associations of HP infection with ageing and DNA methylation alterations, it would appear plausible that HP infection might have an impact on the disproportional agieng reflected by DNA methylation age. To investigate this hypothesis, we explored the associations of both HP infection and CAG with AA in a large population-based study of older adults (ESTHER) in Germany.

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MATERIALS AND METHODS

The methods for this study are described in detail in Supplementary Material. Briefly, we selected 1509 older adults (aged 50–75 years) enroled in the ESTHER study between July 2000 and March 2001 and collected their socio-demographic characteristics, lifestyle factors and health status by standardised questionnaires (Supplementary Figure 1) (Gao *et al*, 2016a). Participants were categorised into three groups according to the serostatus of immunoglobulin G (IgG) antibodies and antibodies specific to the cytotoxin-associated gene A (CagA): infected by less virulent strains (IgG+/CagA−), infected by virulent strains (IgG+/CagA+) and non-infected (IgG−/CagA−) (Chen *et al*, 2016). We excluded 32 participants with IgG−/CagA+ HP, which often reflects past infection. Serum concentrations of pepsinogen (PG) I and II were measured as biomarkers of CAG, and we used the following commonly employed serological classification to define CAG: PG I $<70\,ng\,ml^{-1}$ and PG I/II ratio <3.0. Blood DNA methylation was quantified by Illumina 450K chip and the DNA methylation age of each participant was calculated by the algorithms of Horvath and Hannum *et al* (Hannum *et al*, 2013; Horvath, 2013). AAs were determined as the residuals calculated by the linear regression models of both forms of epigenetic age on chronological age (Gao *et al*, 2016b).

RESULTS

Table 1 summarises characteristics of the 1477 eligible participants. Average chronological age was ~62 years. Hannum *et al* methylation ages were higher than chronological age and age computed by Horvath's approach. Both forms of DNA methylation age and chronological age were highly correlated with each other (Supplementary Figure 2). The prevalence of any HP and CagA+ HP infection was 51.8% and 26.7%, respectively, and around 12% participants had CAG (mild or severe). HP infection status and serostatus were significantly associated with both AAs (Table 2; *P*-values <0.05). Participants infected with virulent strains (CagA+) had the highest AAs, followed by those infected with less virulent strains (CagA−). Similarly, both AAs demonstrated positive correlations with the CAG status and severity of CAG, which was though statistically significant only for the AA based on the algorithm by Hannum *et al* (Table 2; *P*-values $=0.015/0.024$).

We further examined the associations of AAs (outcomes) with HP infection status or CAG severity (independent variables) by three linear regression models, increasingly controlling for potential covariates. HP infection, infection with CagA+ strains and severe CAG were significantly associated with both AAs after adjustment for age, sex and the leucocyte distribution (Table 3; all *P*-values <0.05). Although in the fully adjusted model, associations of the AA according to Horvath's algorithm with HP infection/severe CAG were slightly attenuated (*P*-values $=0.05$), additional adjustment for other covariates did not alter the patterns in any relevant manner. However, CAG (yes/no) was only associated with the AA according to Hannum's algorithm, but not the AA according to Horvath's algorithm. This pattern remained essentially unchanged in the sensitivity analysis in which we adjusted for both HP infection status and the CAG status simultaneously (*P*-value of CAG for $AA_{Horvath}=0.118$; *P*-value of CAG for $AA_{Hannum}=0.012$). Overall, we observed that HP infection, infection of CagA+ strains and severe CAG were associated with the DNA methylation-defined AA. In the fully adjusted model, these risk factors were associated with an increase in DNA methylation age by ~0.4, 0.6 and 1 year, respectively.

Table 1. Population characteristics of ESTHER study[a]

Characteristic	
N	1477
Age (years)	62.0 (6.5)
Methylation age 1 (Horvath, years)	62.2 (7.3)
Methylation age 2 (Hannum, years)	68.2 (7.1)
Gender (male)	495 (50.6%)
HP serostatus	
IgG−/CagA−	712 (48.2%)
IgG+/CagA−	371 (25.1%)
IgG+/CagA+	394 (26.7%)
Severity of CAG[b]	
Non-atrophic	1304 (88.3%)
Mild atrophic	75 (5.1%)
Severe atrophic	98 (6.6%)
Smoking status	
Current smoker	276 (18.7%)
Former smoker	499 (33.8%)
Never smoker	702 (47.5%)
Body mass index[c]	
Underweight or normal weight (<25.0)	399 (27.1%)
Overweight (25 to <30)	685 (46.5%)
Obese (⩾30.0)	390 (26.5%)
Alcohol consumption[d]	
Abstainer	471 (34.3%)
Low	804 (58.5%)
Intermediate	78 (5.7%)
High	21 (1.5%)
Physical activity[e]	
Inactive	294 (19.9%)
Low	673 (45.6%)
Medium or high	510 (34.5%)
Education[f]	
⩽9 years	1071 (74.1%)
10–11 years	224 (15.5%)
⩾12 years	150 (10.4%)
Prevalence of CVD[g] (yes)	265 (17.9%)
Prevalence of diabetes[h] (yes)	230 (15.7%)
Prevalence of cancer (yes)	88 (6.0%)

Abbreviations: CAG = chronic atrophic gastritis; CVD = cardiovascular disease; HP = *Helicobacter pylori*.
[a]Mean values (s.d.) for continuous variables and *n* (%) for categorical variables.
[b]Categories defined as follows: non-atrophic (PG I ⩾ $70\,ng\,ml^{-1}$ and PG I/II ratio ⩾3.0), mild atrophic ($20\,ng\,ml^{-1}$ ⩽PG I $<70\,ng\,ml^{-1}$ and PG I/II ratio <3.0), severe atrophic (PG I $<20\,ng\,ml^{-1}$ and PG I/II ratio <3.0).
[c]Data missing for three participants.
[d]Data missing for 103 participants, respectively. Categories defined as follows: abstainer, low (women: 0 to $<20\,g\,d^{-1}$, men: 0 to $<40\,g\,d^{-1}$), intermediate (20 to $<40\,g\,d^{-1}$ and 40 to $<60\,g\,d^{-1}$, respectively), high (⩾$40\,g\,d^{-1}$ and ⩾$60\,g\,d^{-1}$, respectively).
[e]Categories defined as follows: inactive (<1 h of physical activity per week), medium or high (⩾2 h of vigorous or ⩾2 h of light physical activity per week), low (other).
[f]Data missing for 32 participants.
[g]Data missing for 16 participants.
[h]Data missing for one participant.

DISCUSSION

In summary, we found HP infection, in particular, infection with CagA+ HP strains, to be associated with accelerated ageing. The underlying mechanisms are not yet to be elaborated by further research. A plausible pathological mechanism might be the oxidative damage triggered by the reactive oxygen species that resulted from the inflammation induced by the HP infection (Farinati *et al*, 2008). The damage caused by oxidative stress has been commonly recognised as a contributor to the functional decline related to ageing (Hekimi *et al*, 2011) and has been found

Table 2. Distributions of age accelerations based on *Helicobacter pylori* (HP) infection and severity of chronic atrophic gastritis (CAG)

		Age acceleration (Horvath)		Age acceleration (Hannum)	
Categories	***N***	**Median (interquartile range)**	***P*-value[a]**	**Median (interquartile range)**	***P*-value[a]**
HP infection (yes/no)			**0.004**		**0.007**
No (IgG–)	712	−0.635 (−3.558 to 2.660)		−0.424 (−3.282 to 2.432)	
Yes (IgG+)	765	0.218 (−2.974 to 3.383)		0.185 (−2.528 to 3.019)	
HP serostatus			**0.006**		**0.014**
IgG–/CagA–	712	−0.635 (−3.558 to 2.660)		−0.424 (−3.282 to 2.432)	
IgG+/CagA–	371	−0.249 (−3.107 to 2.979)		−0.089 (−2.541 to 2.784)	
IgG+/CagA+	394	0.756 (−2.894 to 3.557)		0.450 (−2.470 to 3.102)	
HP infection risk			**0.005**		**0.014**
Low (CagA–)	1083	−0.454 (−3.346 to 2.758)		−0.291 (−3.092 to 2.536)	
High (CagA+)	394	0.756 (−2.894 to 3.557)		0.450 (−2.470 to 3.102)	
CAG (yes/no)			0.078		**0.015**
No (non-atrophic)	1304	−0.321 (−3.316 to 2.951)		−0.220 (−2.972 to 2.677)	
Yes (mild/severe atrophic)	173	0.687 (−2.975 to 3.399)		0.614 (−2.576 to 3.600)	
Severity of CAG			0.155		**0.024**
Non-atrophic	1304	−0.321 (−3.316 to 2.951)		−0.220 (−2.972 to 2.677)	
Mild atrophic	75	0.307 (−3.275 to 3.168)		0.151 (−3.058 to 3.243)	
Severe atrophic	98	1.208 (−2.975 to 3.463)		0.897 (−2.230 to 3.927)	

Abbreviations: CAG = chronic atrophic gastritis.
[a]Tested by Wilcoxon test (binomial variables) or Kruskal–Wallis test (variables with three categories).
Bold values indicate that this *P*-value is less than 0.05 and therefore is statistically significant.

Table 3. Associations of *Helicobacter pylori* (HP) infection and severity of chronic atrophic gastritis (CAG) with age acceleration

		Age acceleration (Horvath)						Age acceleration (Hannum)					
		Model 1[a]		**Model 2[b]**		**Model 3[c]**		**Model 1[a]**		**Model 2[b]**		**Model 3[c]**	
Categories	***N***	**Beta (s.d.)**	***P*-value**	**Beta (s.d.)**	***P*-value**	**Beta (s.d.)**	***P*-value**	**Beta (s.d.)**	***P*-value**	**Beta (s.d.)**	***P*-value**	**Beta (s.d.)**	***P*-value**
HP infection (yes/no)													
No (IgG–)	712	Ref		Ref		Ref		Ref		Ref		Ref	
Yes (IgG+)	765	0.613 (0.242)	**0.011**	0.376 (0.254)	**0.049**	0.366 (0.255)	0.050	0.538 (0.209)	**0.010**	0.432 (0.217)	**0.026**	0.450 (0.218)	**0.039**
HP serostatus													
IgG–/CagA–	712	Ref		Ref		Ref		Ref		Ref		Ref	
IgG+/CagA–	371	0.411 (0.297)	0.166	0.371 (0.312)	0.906	0.063 (0.313)	0.841	0.399 (0.256)	0.119	0.290 (0.267)	0.277	0.312 (0.269)	0.246
IgG+/CagA+	394	0.803 (0.290)	**0.006**	0.697 (0.307)	**0.023**	0.652 (0.307)	**0.034**	0.668 (0.251)	**0.008**	0.567 (0.267)	**0.031**	0.581 (0.263)	**0.028**
HP infection risk													
Low (CagA–)	1083	Ref		Ref		Ref		Ref		Ref		Ref	
High (CagA+)	394	0.659 (0.271)	**0.015**	0.684 (0.288)	**0.018**	0.631 (0.288)	**0.029**	0.528 (0.234)	**0.024**	0.468 (0.245)	**0.042**	0.475 (0.247)	**0.045**
CAG (yes/no)													
No (non-atrophic)	1304	Ref		Ref		Ref		Ref		Ref		Ref	
Yes (mild/severe atrophic)	173	0.648 (0.373)	0.083	0.586 (0.390)	0.134	0.637 (0.391)	0.103	0.847 (0.322)	**0.009**	0.790 (0.332)	**0.018**	0.876 (0.334)	**0.009**
Severity of CAG													
Non-atrophic	1304	Ref		Ref		Ref		Ref		Ref		Ref	
Mild atrophic	75	0.150 (0.547)	0.784	0.123 (0.566)	0.828	0.181 (0.569)	0.750	0.522 (0.472)	0.268	0.517 (0.482)	0.284	0.637 (0.487)	0.191
Severe atrophic	98	1.030 (0.483)	**0.033**	1.003 (0.508)	**0.049**	0.994 (0.508)	0.050	1.095 (0.417)	**0.009**	1.064 (0.433)	**0.014**	1.063 (0.434)	**0.015**

Abbreviation: CAG = chronic atrophic gastritis; s.d.=standard deviation.
[a]Adjusted for age, sex and the leucocyte distribution (Houseman algorithm).
[b]Model 1 plus smoking status, body mass index (BMI, $kg\,m^{-2}$, underweight (<18.5, <1% of the study population) or normal weight (18.5 to <25), overweight (25 to <30), obese (⩾30)), alcohol consumption (abstainer, low (women: 0 to $<20\,g\,d^{-1}$, men: 0 to $<40\,g\,d^{-1}$), intermediate (20 to $<40\,g\,d^{-1}$ and 40 to $<60\,g\,d^{-1}$, respectively), high (⩾$40\,g\,d^{-1}$ and ⩾$60\,g\,d^{-1}$, respectively)), education levels (⩽9 years, 10–11 years, ⩾12 years) and physical activity (inactive (<1 h of physical activity per week), medium or high (⩾2 h of vigorous or ⩾2 h of light physical activity per week), low (other)).
[c]Model 2 plus prevalence of CVD, diabetes and cancers.
Bold values indicate that this P-value is less than 0.05 and therefore is statistically significant.

to be associated with ageing-related DNA methylation changes in whole blood samples (Gao *et al*, 2017).

Although we controlled for a large number of potential confounders in multivariate analyses, a causal relationship cannot be derived from this cross-sectional epidemiological study. Nevertheless, a causal relationship appears to be plausible due to a variety of reasons. First, HP infection is typically acquired in early childhood (Zhang *et al*, 2012), a period during which the foundation of age acceleration seems to be laid (Simpkin *et al*, 2016), and clearly precedes the pattern of age acceleration observed among older adults in our study, despite its cross-sectional design. Second, both HP infection and CAG have been associated with a number of age-related chronic diseases, including but not restricted to gastric cancer, such as CVD and extra-gastric diseases (e.g.,

colonic and pancreatic cancer; Franceschi *et al*, 2009; Chen *et al*, 2016). Further research should address the association between HP infection and DNA methylation age at various ages across the lifespan, ideally in a large cohort study in which temporal relationship between the occurrence of HP infection and AA could be established by repeated measurements of both factors. Another question of major scientific interest and potential major public health relevance might be to what extent AA associated with CagA + HP infection could be reversible after HP eradication.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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