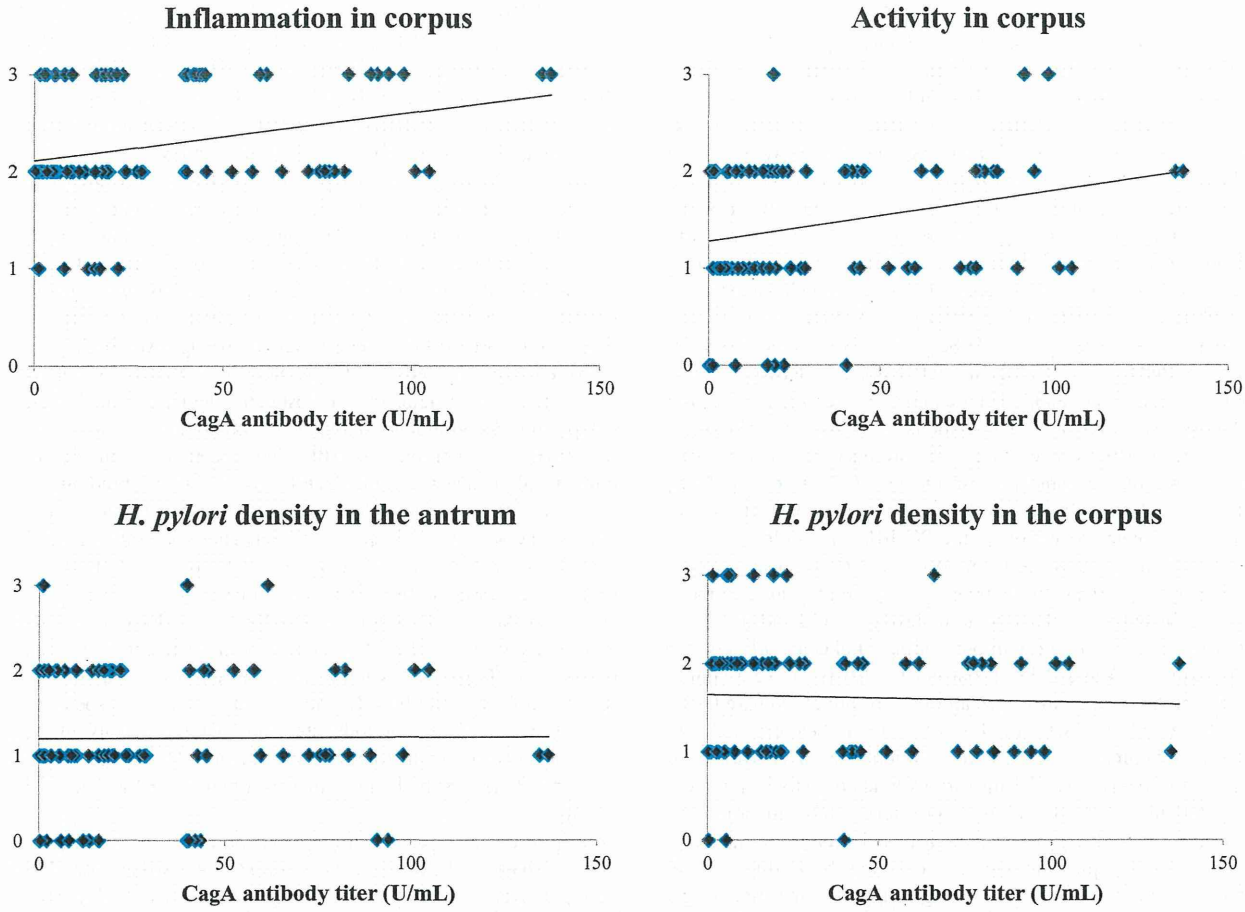

Figure 2 The correlation between serum CagA antibody titer and gastric mucosal inflammation, bacterial density.

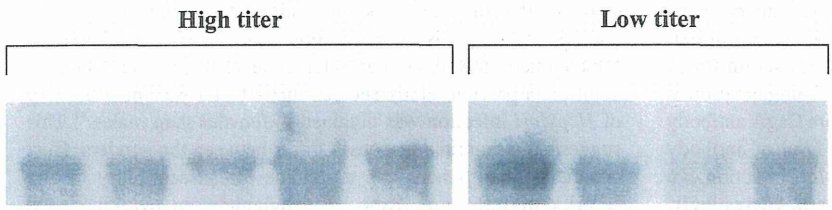

Figure 3 CagA protein expression profile. Bacterial protein was extracted in the strains from the patients with serum CagA antibody negative/low pepsinogen (PG) II and serum CagA antibody positive/high PG II, respectively. CagA protein expression was examined by Western blot.

the bacterial CagA expression level, bacterial CagA expression levels were examined by immunoblot. We selected four samples from serum CagA antibody negative/low PG II level and five samples from serum CagA antibody positive/high PG II level. As a result, there was no difference of CagA expression level (Fig. 3). Even in the strain isolated from patients with serum CagA antibody negative/low PG II level, the CagA expression was found, and there was no significant difference compared with that of serum CagA antibody positive/high PG II level. This suggests that low CagA expression level in the bacteria does not contribute to the low serum CagA antibody titer.

Discussion

In East Asian countries, different CagA seropositivity has been reported despite almost all *H. pylori* possessing *cagA*. CagA seropositivity in gastritis ranged from 53.7% to 81.1%, even in Japan.[17,18] In our meta-analysis, CagA seropositivity was associated with gastric cancer even in East Asian countries, although the odds ratio in East Asian countries was smaller than in studies that included Western countries.[19] Furthermore, even in the *H. pylori*-negative population, the presence of anti-CagA antibodies increases the risk of gastric cancer.[19] This evidence confirms that

CagA antibodies can potentially remain positive for a longer period of time than the anti-*H. pylori* antibody.[22,23] Accordingly, anti-CagA antibody was related to gastric cancer in both *H. pylori*-positive and -negative populations in East Asian countries.

Serum PG has been found to be a marker of gastric mucosal status including atrophy and inflammation.[24] There are two forms of PG: PG I and PG II, and both are produced by the chief and mucus neck cells in the gastric fundus and corpus. PG II is also produced by the pyloric glands in the antrum and Brunner's glands in the proximal duodenum. Although atrophy is usually diagnosed by endoscopic biopsy, there is a significant potential sampling errors in identifying atrophy by random biopsy because atrophy of gastric mucosa could be patchy. On the other hand, PG was reported to be used as a surrogate marker for gastric mucosal status.[25] Serum PG I and PG II are known to increase by *H. pylori* infection. However, as PG II exhibits a greater raise relative to PG I, the PG I/II ratio decrease in the presence of *H. pylori*. After that, as the fundic gland mucosa reduces, PG I levels gradually decrease, whereas PG II levels remain fairly constant. As the result, a stepwise reduction of the PG I/II ratio is closely correlated with the progression from normal gastric mucosa to extensive atrophic gastritis. In the present study, serum CagA antibody was significantly correlated with the levels of PG I and II, but not PG I/II ratio. Consistent with our findings, Fukuda *et al.* reported that serum PG I and II level, but not PG I/II ratio, were significantly higher in serum CagA antibody positive compared with negative children.[26] Serum PG was reported to be correlated with gastric inflammatory score.[27] In addition, the *cagA* status was reported to be associated with various kinds of cytokines including interleukin-8 (IL-8) and may cause severe inflammation in the stomach.[28] It is also possible that gastritis increases permeability of the gastric epithelial surface, enabling back diffusion of PGs after secretion.[27] These findings suggest that serum CagA antibody titer was associated with gastric inflammation, but not atrophy.

Shimoyama *et al.* reported that inflammation in the antrum and the corpus was more significant in serum CagA antibody positive when they examined the presence of serum CagA antibody by immunoblot.[29] In the present study, although there were no significant differences of each histological score between serum CagA antibody positive and negative group, the mucosal inflammation in the corpus was significantly correlated with serum CagA antibody titer. This finding also supported that different level of antibody production from lymphocytes induced by *H. pylori* infection can contribute to the various serum CagA antibody level. Interestingly, positive correlation between the inflammatory score and serum CagA antibody titer was found only in the corpus but not in the antrum. Corpus dominant gastritis rather than antrum dominant gastritis was a risk factor to develop gastric ulcer and gastric cancer.[3,30] In addition, even when only serum CagA antibody positive group was selected, serum CagA antibody titer was significantly correlated with inflammation and activity in the corpus. Therefore, antibody titer rather than the presence of antibody can be a useful marker for advanced inflammation in the stomach in Japan. This suggests that serum CagA antibody titer might be an available marker to predict a gastric cancer in Japan. It has also been reported that measurement of serum levels of C-reactive protein (CRP) using a high-sensitivity assay (hs-CRP) can reveal subclinical inflammatory states that may reflect vascular inflammation.[31] Recent report showed that the mean serum level of hs-CRP was significantly higher in *H. pylori*-positive group than *H. pylori*-negative group, although the level of hs-CRP was not different between CagA antibody positive and negative group in Iran.[32] It is better to examine the association between serum CagA antibody and hs-CRP in Japan in the further study.

In our study, in spite of *cagA* positive by PCR, the prevalence of serum CagA antibody was 75.0%, which was consistent with previous studies from Japan.[17,33] The *cagA* gene is located at one end of the *cag* pathogenicity island (PAI), an approximately 40-kbp region that is thought to have been incorporated into the *H. pylori* genome by horizontal transfer from an unknown source.[34] The *cag* PAI encodes a type IV secretion system, through which CagA is delivered into host cells.[35,36] CagA has been reported to interact with various target molecules in host cells; the best studied is the cytoplasmic Src homology 2 domain of Src homology 2 phosphatase (SHP-2). Mutations of SHP-2 have been found in various human malignancies and mice that lacked the SHP-2-binding site developed hyperplastic antral tumors,[37] indicating that SHP-2 plays an important role in gastric cancer. Therefore, other gene(s) except for *cagA* in *cag* PAI can contribute to the difference of serum CagA antibody titer. However, almost of case was *cag* PAI positive in Japan.[29] Therefore, it is unlikely that diversity of *cag* PAI can contribute to the difference of serum CagA antibody titer. Furthermore, CagA expression pattern was not associated with the serum CagA antibody titer. In addition, there was no association between serum CagA antibody titer and bacterial density in the antrum and corpus by histological examination. This suggests that low serum CagA antibody titer cannot attribute to the low bacterial density.

Therefore, our findings suggest that host and environmental factors can affect the difference of serum CagA antibody titer. For example, even when healthy volunteers were infected with same strains, they showed different histological score.[38] Therefore, host recognition can be associated with the difference of serum CagA antibody titer. We found that serum CagA antibody positive rate was significantly higher in female than male irrespective of the status of PG. In general, estrogen stimulates immune responses, and testosterone is immunosuppressive.[39] *H. pylori*-infected female mice showed the higher IgG2c levels than male mice.[40] In addition, a previous study showed that a better vaccine efficiency of *H. pylori* infection was obtained in females than males.[41] This suggests that immune responses differ between the genders. Host genetic polymorphisms can determine the susceptibility to and severity of infection.[2] Especially, inflammatory cytokine gene polymorphisms (*IL-1* gene cluster, *tumor necrosis factor-alpha*, *IL-10*, and *IL-8*) have been reported to be correlated with gastric cancer.[42–47] In addition, environmental factors such as diet (e.g. salt intake) can also affect the gastric cancer incidence.[48] Loh *et al.* reported the increased expression of *cagA* in response to high-salt conditions.[49] Furthermore, they showed that co-culture of gastric epithelial cells with *H. pylori* in high-salt conditions resulted in the increased tyrosine-phosphorylated CagA and increased secretion of IL-8 by the epithelial cells compared with low-salt conditions. These findings provide important insights into mechanisms through which high-salt diets increase the risk for gastric cancer among subjects infected with *cagA*-positive *H. pylori*. Further studies using host and environmental information are necessary to elucidate the contributing factors for serum CagA antibody titer.

However, we should keep a caution for the difference of serum CagA antibody titer examined by ELISA. We found a significant heterogeneity in a meta-analysis.[19] This heterogeneity appeared to result from the use of different populations or different methods, or from differences in the antigens used to detect anti-CagA antibodies. We previously examined the relationship between serum CagA antibody and gastric cancer in a Japanese population using two different recombinant CagA antigens.[18] CagA seropositivity was 82% by OraVax antigen and 72% by Chiron antigen, irrespective of the existence of gastric cancer, when determining the cut-off value by the population living in the same region (Kyoto, Japan). This suggests that numerical results from studies using different antigens and different protocols may not be comparable.[50,51] Because many recombinant CagA as coating antigen in ELISA system were derived from European strain, recombinant CagA derived from East Asian strain may be proper in East Asian countries. The CagA can be of two types: East Asian-type CagA and Western-type CagA according to the difference of amino acid sequences of the C-terminal of CagA.[52] Individuals infected with East Asian-type CagA strains reportedly have an increased risk of peptic ulcer or gastric cancer compared with individuals with Western-type CagA strains.[53,54] East Asian-type CagA or Western-type CagA status may also affect the serum CagA antibody titer and/or different sensitivity of assay. At present, there are no reports that examine the prevalence of East Asian-type CagA-specific antibody in sera. Yasuda *et al.* reported the development of monoclonal antibody against East Asian-type CagA for developing a sandwich-ELISA system.[55] However, this is the system for detecting East Asian-type CagA strains but not serum antibody. To detect serum East Asian-type CagA-specific antibody, the development of an ELISA assay using East Asian-type CagA-specific antigen will be required.

In conclusion, our study revealed that high serum CagA antibody titer was significantly correlated with PG I, PG II, and inflammation in the corpus. Therefore, subjects with higher serum CagA antibody titer can be considered as high-risk population for the development of gastric cancer from the point of strong gastric inflammatory response even in Japan.

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Prevalence of *Helicobacter pylori* Infection by Birth Year and Geographic Area in Japan

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H. pylori, prevalence, gastric cancer, birth cohort.

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Abstract

Background: *Helicobacter pylori* (*H. pylori*)-related diseases are responsible for a tremendous amount of morbidity and mortality in Japan. We estimated the prevalence of *H. pylori* infection by sex, birth year, and geographic area among Japanese adults.

Materials and Methods: This cross-sectional study included 14,716 subjects aged 20 years or more who underwent a health checkup between May 1997 and March 2013 in seven geographic areas throughout Japan. Relevant information on the demographics and status of *H. pylori* infection was retrieved from the electronic database. The univariate log-binominal regression model was used to estimate the prevalence of *H. pylori* infection, taking birth year into consideration. The multivariate log-binominal regression model was used to compare the prevalence of *H. pylori* infection between seven geographic areas.

Results: The overall prevalence of *H. pylori* infection was 37.6% in women and 43.2% in men. Among seven geographic areas, Hokkaido showed the lowest prevalence (29.4%), while Yamagata Prefecture represented the highest (54.5%). The prevalence of H. pylori infection was highest in the 1940–1949 birth cohort and then decreased in the ensuing birth cohorts; the risk ratio (RR) was 0.85 (95% confidence interval (CI) 0.84–0.87) for changes in the 10-year birth cohort. Individuals in Yamagata Prefecture had the highest RR of acquiring H. pylori infection in all three birth cohorts (RR = 1.53 for 1940, RR = 1.69 for 1950, and RR = 1.85 for 1960) when compared with those in Hokkaido.

Conclusions: The prevalence of *H. pylori* infection increases with age and exhibits geographic variation in Japan. There has been a striking decrease in the prevalence of *H. pylori* infection, especially in younger Japanese populations.

An estimated half the population of the world is infected with *Helicobacter pylori* (*H. pylori*). *H. pylori* infection causes digestive diseases such as gastro/duodenal ulcers and chronic gastritis and increases the risk of noncardiac gastric cancer [1]. *H. pylori*-related diseases are responsible for a tremendous amount of morbidity and mortality in Japan. One of the more striking features of *H. pylori* infection is geographic variation, with developing countries having a much higher prevalence compared with developed countries [2]. Although

Japan is a developed country, both the prevalence of *H. pylori* infection and incidence of gastric cancer are among the highest in the world.

The prevalence of *H. pylori* infection used to be very high in asymptomatic Japanese. The most-cited study, published in 1992, showed that the prevalence of serum anti-*H. pylori* antibody increased with age and that individuals born before 1950 had a prevalence as high as 70–80% [3]. However, as with other developed countries, the prevalence in Japan has been continuously decreasing over the past several decades. Using random samples collected at three points in time, Fujisawa et al. reported that the overall seroprevalence of *H. pylori* was 72.7% in 1974, 54.6% in 1984, and 39.3% in 1994 [4]. This finding suggested that a marked decrease in *H. pylori* infection had occurred in Japan.

Despite many studies of *H. pylori* prevalence in selected areas of Japan [5–9], it remains unclear whether its prevalence differs across geographic areas. Moreover, very few studies have reported the prevalence of *H. pylori* infection by birth year. We believe that the epidemiology of *H. pylori* infection sheds light on the geographic differences in the prevalence of *H. pylori*-associated diseases. Using data collected from a large number of health checkup participants, we estimated the updated prevalence of *H. pylori* infection among Japanese adults by sex, birth year, and geographic area.

Methods

Study Population

This is a cross-sectional, multi-institutional study of *H. pylori* prevalence in Japanese adults. Our study included individuals aged 20 years or more who underwent a health checkup provided by their municipal government or private health screening center/clinic, between May 1997 and March 2013, in Hokkaido (Yubari), Tokyo, and nine other prefectures (Aomori, Yamagata, Gunma, Aichi, Shiga, Okayama, Hiroshima, Kagawa, and Oita) throughout Japan. Of these areas, data collected from outpatients residing in four areas (Tokyo, Okayama, Hiroshima, and Oita) were excluded, and thus, the remaining seven areas were eligible for the present study. In addition, individuals with a history of *H. pylori* eradication therapy were excluded. Information on identification number, sex, birth date, type of health checkup, inspection date, *H. pylori* infection status, history of *H. pylori* eradication therapy, and serum pepsinogen (I and II) levels were retrieved from the electronic database. *H. pylori* infection was determined using serologic, urinary, or stool antigen tests. The serologic test was performed for quantifying *H. pylori* -IgG antibody using ELISA-kit "E-plate Eiken *H. pylori* antibody" (Eiken Kagaku, Tokyo, Japan). The recommended cutoff point of antibody titers was used to define *H. pylori* infection. A stool antigen test was performed using TFB Meridian HpSA ELISA2, and the urine antibody test performed using RAPIRUN (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). If an individual underwent a diagnostic test for *H. pylori* periodically, we selected the result of the first test. Furthermore, for individuals who had undergone more than one diagnostic test, results of the serologic test were given top priority. Our study was approved by the Ethics Committee of Hokkaido University.

Statistical Method

Continuous variables were summarized as the mean (standard deviation), and categorical variables were presented as numbers and percentages. The prevalence of *H. pylori* infection was estimated using a univariate log-binomial regression model that included birth year as a factor [10]. The risk ratio (RR) represented the comparison of the proportion of being *H. pylori*—positive in the case of a 10-year increase in birth cohort. Using multivariate log-binomial regression models, we compared the prevalence of *H. pylori* infection for the seven areas in three birth cohorts (1940, 1950, and 1960). Four factors (area, birth year, sex, and clinical test method) and the interaction term between area and birth year were added in the models. The reference group for area, sex, and clinical test method is Hokkaido, female, and health checkup provided by municipal government, respectively.

All tests were two-sided, and p values less than 0.05 were considered to indicate statistical significance. Statistical analyses were carried out using SAS 9.3 (SAS institute Inc., Cary, NC, USA).

Results

Figure 1 shows the flowchart of selection of the study subjects. After the exclusions described in Methods, data collected from 14,716 people were eligible for the present analysis. Table 1 presents the characteristics of the study population. Of the 14,715 people who underwent diagnostic tests for *H. pylori* infection, data on the serologic test were available for 11,470 (77.9%) people. The status of *H. pylori* infection was determined by a urine antibody test in Hokkaido (Yubari) and by a stool antigen test in Aichi Prefecture. The overall prevalence of *H. pylori* infection was 37.6% in women and 43.2%

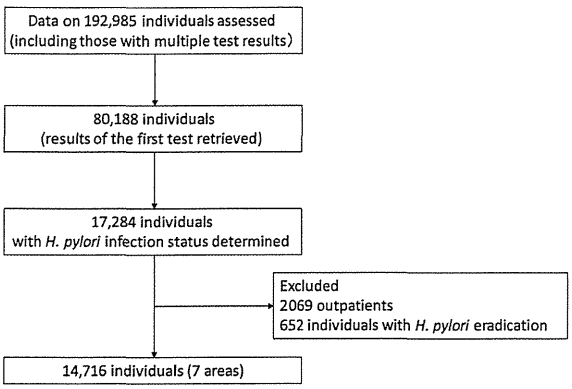

Figure 1 A flowchart of selection of study subjects.

in men. The mean birth year was 1950 among *H. pylori*-positive subjects and 1956 among *H. pylori*-negative subjects. Among seven geographic areas, Hokkaido showed the lowest prevalence of *H. pylori* infection (29.4%), while Yamagata Prefecture represented the highest (54.5%). Aomori and Shiga also had a high prevalence compared with other prefectures. The prevalence of *H. pylori* infection was slightly higher among individuals who underwent a health checkup provided by the municipal government compared with that among those who underwent a health checkup provided by private health screening centers/clinics.

The results of univariate analysis showed that the prevalence of *H. pylori* infection was highest in the 1940–1949 birth cohort and then decreased in the ensuing birth cohorts; the RR was 0.85 (95%CI 0.84–0.87) for changes in the 10-year birth cohort (Fig. 2). Figure 3 shows the results of multivariate log-binomial regression analysis. Individuals in Yamagata Prefecture had the highest RR of acquiring *H. pylori* infection of all three birth cohorts (RR = 1.53 for 1940, RR = 1.69 for 1950, and RR = 1.85 for 1960) when compared with those in Hokkaido.

Discussion

Despite the retrospective nature of our study, we collected a large amount of updated data on the status of *H. pylori* infection from healthy checkup participants who resided in various geographic areas in Japan. We found that the prevalence of *H. pylori* infection showed geographic variations. We also examined the effect of birth year on the prevalence of *H. pylori* infection and found a clear birth cohort effect that was occurring in Japan: Individuals who belonged to young birth cohorts had a decreased prevalence compared with old birth cohorts.

One striking feature of *H. pylori* is geographic variation. Although differences in prevalence have been observed in different geographic regions within a coun-

Table 1 Characteristics of the study subjects

Characteristics	Category	*H. pylori* -positive (n = 5879)	*H. pylori*-negative (n = 8837)	Total (n = 14716)
Sex	Women	3184 (37.6%)	5293 (62.4%)	8477 (57.6%)
	Men	2695 (43.2%)	3544 (56.8%)	6239 (42.4%)
Birth year	Mean (SD)	1950 (11)	1956 (16)	1954 (14)
Geographic area	Hokkaido	420 (29.4%)	1008 (70.6%)	1428 (9.7%)
	Aomori	389 (49.7%)	393 (50.3%)	782 (5.3%)
	Yamagata	1969 (54.5%)	1646 (45.5%)	3615 (24.6%)
	Gunma	1586 (32.3%)	3328 (67.7%)	4914 (33.4%)
	Aichi	684 (30.6%)	1553 (69.4%)	2237 (15.2%)
	Shiga	664 (51.2%)	634 (48.8%)	1298 (8.8%)
	Kagawa	167 (37.8%)	275 (62.2%)	442 (3%)
Diagnostic method	Serology	4963 (43.3%)	6507 (56.7%)	11470 (77.9%)
	Urine antibody	232 (23%)	776 (77%)	1008 (6.9%)
	Stool antigen test	684 (30.6%)	1553 (69.4%)	2237 (15.2%)
Type of health checkup	Health checkup provided by municipal government	4153 (38.1%)	6752 (61.9%)	10905 (74.1%)
	Health checkup provided by private health screening center/clinic	1726 (45.3%)	2085 (54.7%)	3811 (25.9%)

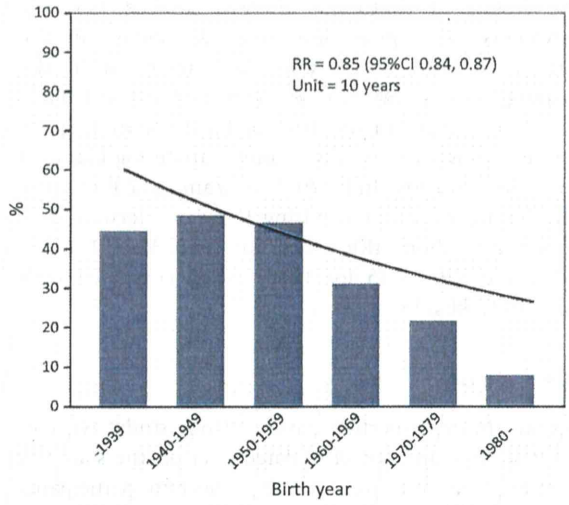

Figure 2 The effect of birth year on the prevalence of *H. pylori* infection: a univariate analysis.

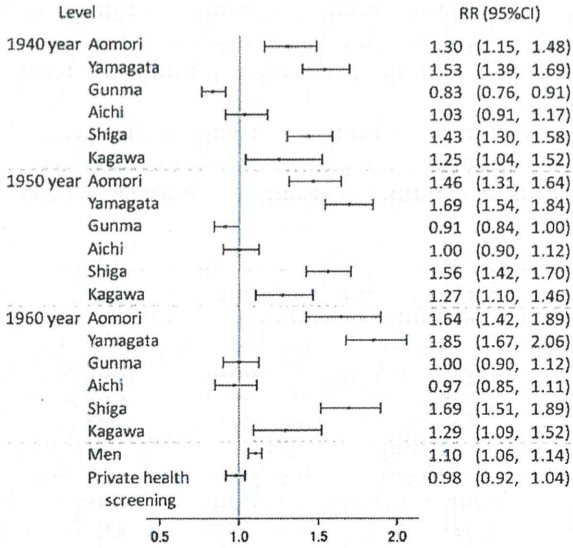

Figure 3 The effect of area, birth year, sex, and clinical test method on *H. pylori* prevalence : a multivariate analysis. Bars represent the RR with a 95% CI for *H. pylori* prevalence. The RR is estimated by the use of the multiple log-binominal regression model including area, birth year, sex, and clinical test method as factors and the interaction term between area and birth year. The prevalence of *H. pylori* infection in each area (Aomori, Yamagata, Gunma, Aichi, Shiga, and Kagawa) is compared to that in Hokkaido at 1940, 1950, and 1960 years. The reference group for area, sex, and clinical test method is Hokkaido, women, and health checkup provided by municipal government, respectively. RR, multivariate-adjusted risk ratio; CI, confidence interval.

try [11], few studies have examined the effect of geographic areas on *H. pylori* prevalence in Japan. Our finding showed that geographic variation in the prevalence of *H. pylori* infection existed in Japan, with Yamagata Prefecture having the highest prevalence among the seven prefectures examined. Previous studies have indicated that genetic diversity of *H. pylori* strains, socioeconomic status, and environmental factors may play a role in *H. pylori* infection [12–15], contributing to the geographic variation. Given that *H. pylori* colonization occurred mainly in children under 5 years old and persisted in one's whole life without eradication, socioeconomic status during childhood may be the major contributing factor underlying of *H. pylori* infection. In Yamagata Prefecture, three generations under one roof was common and this may be, in part, associated with a high prevalence of *H. pylori* infection. In contrast, Hokkaido (Yubari) had a complete water supply system since 1950s, which may account for the low prevalence. Another important factor is the genetic diversity of *H. pylori* strains. It has been shown that the virulence factors of *H. pylori*, such as CagA and Vac A, varied across regions in the world [12]. The majority of the *H. pylori*-positive subjects in Japan possess CagA, while those in other developed countries are colonized by an almost equal proportion of CagA-positive and CagA-negative strains [12]. CagA-positive *H. pylori* strains induce more intense inflammation in the stomach, and individuals with CagA-positive *H. pylori* strains had a significantly increased risk of gastric cancer [12]. Further studies are needed to address the question of which factor is most important in determining the geographic variation.

In this study, the prevalence of *H. pylori* infection increased with age. Our findings confirmed that birth cohorts have different risks of acquiring *H. pylori* infection. The birth cohort effects reflect a decrease in the rate of acquisition of *H. pylori* infection in successive generations of children as sanitation and living standards improved. This phenomenon has been consistently observed in cross-sectional studies of *H. pylori* infection [13,16,17]. One thing to note here is that the prevalence of *H. pylori* infection among individuals who were born before 1940 was lower than expected. There are several possible reasons. The first possibility is that in the elderly, *H. pylori* may disappear with the progression of gastric atrophy. Another possibility is that incidental *H. pylori* eradication might have occurred as a result of the widespread use of antibiotics in the elderly for the treatment of diseases such as upper respiratory diseases.

A decline in the age-standardized incidence of gastric cancer has been noted in many countries, including Japan. An examination of secular trends in *H. pylori* prevalence and gastric cancer incidence showed that the decline in *H. pylori* prevalence was in parallel with

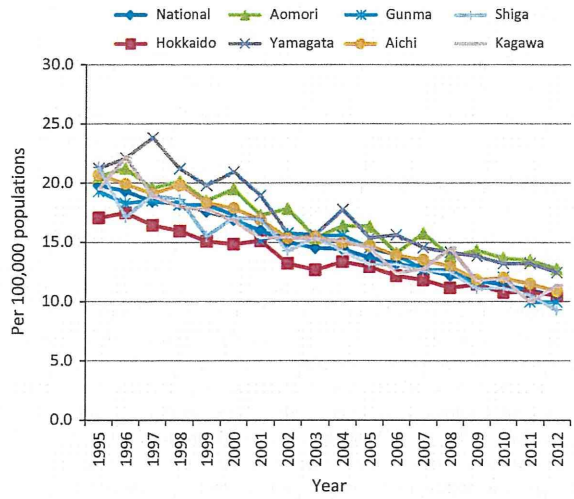

Figure 4 Age-adjusted mortality rates of gastric cancer in selected areas in Japan; 1995–2012. Source: Vital statistics, provided by Center for Cancer Control and Information Services, National Cancer Center, Japan. Website access: http://ganjoho.jp/professional/statistics/statistics.html#05

a decline in gastric cancer incidence [18]. Using a population-based microsimulation model, Yeh et al. estimated that approximately 50% of the observed decrease in distal gastric cancers from 1978 to 2008 in the United States could be attributable to the decline in *H. pylori* prevalence [19]. Similarly, when comparing the prevalence of *H. pylori* infection and age-adjusted mortality rates of gastric cancer in seven areas, we found that *H. pylori* prevalence generally correlated with gastric cancer mortality rates. Although the age-adjusted mortality rates have been declining in all seven areas, Aomori and Yamagata Prefectures had consistently higher rates compared with other areas throughout the period from 1995 to 2012(Fig. 4). Apart from *H. pylori* infection, salt intake is an important risk factor for gastric cancer [20]. There has been strong evidence indicating a synergistic effect of *H. pylori* and salt intake for gastric cancer among Japanese [21]. Despite a continuous decrease in salt intake per day, it was still high when compared with other Western countries [22]. We speculate that the high salt intake and high prevalence of *H. pylori* infection may account for the highest gastric cancer mortality rates observed in Yamagata Prefecture.

Our study has several limitations. First, our study included a small number of young people. There has been a marked decrease in the prevalence of *H. pylori* infection in younger generations over the past several decades in Japan. According to a recent survey, the prevalence was only 12.1% among those aged 1–18 years [23]. A clearer picture of the birth cohort effect for *H. pylori* infection would emerge by including data from young generations. Second, heterogeneities would be expected because we collected data in different prefectures. For example, the characteristics of study subjects and the period for which data were collected might differ across areas. The diagnostic tests also varied across areas. These variations may cause uncertainty in precisely estimating *H. pylori* prevalence. Third, data were retrieved from health checkup participants who may represent a health-conscious group. Therefore, the generalizability of our findings to the general Japanese population may be of a concern, and further studies involving a random sample of the general population are warranted.

In summary, the prevalence of *H. pylori* infection increases with age and exhibits geographic variations in Japan. There has been a striking decrease in the prevalence of *H. pylori* infection in younger Japanese populations. If the decline in *H. pylori* prevalence across various age groups continues, gastric cancer incidence is expected to be continuously decreasing in the coming years.

Acknowledgments and disclosures

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Competing interests: the authors have no conflict of interests.

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