Virulence genes of *Helicobacter pylori* in the Dominican Republic

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Although the incidence of gastric cancer in the Dominican Republic is not high, the disease remains a significant health problem. We first conducted a detailed analysis of *Helicobacter pylori* status in the Dominican Republic. In total, 158 patients (103 females and 55 males; mean age 47.1 ± 16.2 years) were recruited. The status of *H. pylori* infection was determined based on four tests: rapid urease test, culture test, histological test and immunohistochemistry. The status of *cagA* and *vacA* genotypes in *H. pylori* was examined using PCR and gene sequencing. The overall prevalence of *H. pylori* infection was 58.9 %. No relationship was found between the *H. pylori* infection rate and the age range of 17–91 years. Even in the youngest group (patients aged <29 years), the *H. pylori* infection rate was 62.5 %. Peptic ulcer was found in 23 patients and gastric cancer was found in one patient. The *H. pylori* infection rate in patients with peptic ulcer was significantly higher than that in patients with gastritis (82.6 versus 54.5 %, $P<0.01$). The *cagA*-positive/*vacA* s1m1 genotype was the most prevalent (43/64, 67.2 %). Compared with *H. pylori*-negative patients, *H. pylori*-positive patients showed more severe gastritis. Furthermore, the presence of *cagA* was related to the presence of more severe gastritis. All CagA-positive strains had Western-type CagA. In conclusion, we found that *H. pylori* infection is a risk factor for peptic ulcer in the Dominican Republic. Patients with *cagA*-positive *H. pylori* could be at higher risk for severe inflammation and atrophy.

INTRODUCTION

Helicobacter pylori is a spiral, Gram-negative bacterium that chronically colonizes the human stomach and is currently recognized as playing a causative role in the pathogenesis of various gastroduodenal diseases, including gastritis, peptic ulcer, gastric cancer and mucosa-associated lymphoid tissue lymphoma (Peek & Blaser, 2002; Suerbaum & Michetti, 2002). Gastric cancer remains a significant health problem, although its incidence greatly varies geographically. Countries can be categorized as high risk (e.g. Japan), intermediate risk (e.g. Vietnam) or low risk (e.g. the United States) for gastric cancer based on the age-standardized

Abbreviations: ASR, age-standardized incidence rate; IHC, immunohistochemistry; OLGA, Operative Link for Gastritis Assessment.

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incidence rate (ASR) of the malignancy (Ferlay *et al.*, 2010) (this information is also available from the International Agency for Research on Cancer; GLOBOCAN2012, http://globocan.iarc.fr/). Although the association between *H. pylori* infection and gastric cancer is well established (Parsonnet *et al.*, 1991; Uemura *et al.*, 2001), a high prevalence of *H. pylori* infection is not always related to a high incidence of gastric cancer. Interestingly, despite the high prevalence of *H. pylori* infection in Africa and South Asia, the incidence of gastric cancer in these areas is much lower than that in other countries; this phenomenon is the so-called African and Asian enigma (Malaty, 2007).

The Dominican Republic is a nation that occupies the western part of an island shared with the nation of Haiti in the Caribbean Sea. The ASRs of gastric cancer in Caribbean countries are reportedly $<10:100\,000$ year^{-1} (http://globocan.iarc.fr/). The ASR of gastric cancer in the Dominican Republic is reportedly $7.3:100\,000$ year^{-1} (http://globocan.iarc.fr/). Although the prevalence of *H. pylori* infection has been determined in several countries with different socioeconomic, cultural and racial makeups (Azevedo *et al.*, 2009; Goh *et al.*, 2011; Tonkic *et al.*, 2012), the prevalence of *H. pylori* infection in the Dominican Republic has not yet been investigated thoroughly. A previous study reported that the age-adjusted seroprevalence of *H. pylori* infection in the Dominican Republic was 62.1 % (Aoki *et al.*, 2004). This finding suggested that the low virulence of *H. pylori* in the Dominican Republic contributed to the low incidence of gastric cancer. However, serological *H. pylori* antibody titres varied greatly depending on the test kit used (Burucoa *et al.*, 2013; Miki, 2011). Furthermore, previous studies of the prevalence of chronic atrophic gastritis in the Dominican Republic were undertaken based on pepsinogen measurements, not histology (Aoki *et al.*, 2004, 2005). Variable cut-off values for pepsinogen I and the pepsinogen I/II ratio were applied in previous studies, and might have affected the sensitivity and specificity of the results (Brenner *et al.*, 2007; Leung *et al.*, 2008). In this study, we determined the infection rate of *H. pylori* in the Dominican Republic using multiple tests: rapid urease test, culture test, histological test and immunohistochemistry (IHC). We also identified the virulence genes of *H. pylori* in the Dominican Republic.

METHODS

Study population. We recruited 158 patients with dyspeptic symptoms (103 females and 55 males; mean age 47.1 ± 16.2 years, range 17–91 years) at the Digestive Disease Center, Dr Luis E. Aybar Health and Hygiene City, Santo Domingo, Dominican Republic, in February 2011. Patients with a history of partial gastric resection or previous treatment for *H. pylori* infection were excluded. Written informed consent was obtained from all participants, and the protocol was approved by the ethics committees of Dr Luis E. Aybar Health and Hygiene City and Oita University Faculty of Medicine, Japan.

During each endoscopy session, four gastric biopsy specimens were obtained (three from the antrum and one from the corpus). The antrum specimens were used for *H. pylori* culture, rapid urease test and histological examination. The corpus specimen was used for histological examination. Peptic ulcer and gastric cancer were identified using endoscopy. Gastritis was diagnosed in the absence of peptic ulcer or gastric malignancy.

Status of *H. pylori* infection. To maximize the diagnostic accuracy, we used four methods for the diagnosis of *H. pylori* infection: rapid urease test, culture test, histological test and IHC. The CLOtest (Kimberly Clark Ballard Medical Products) was used as a rapid urease test to detect the presence of *H. pylori* urease. *H. pylori* culture was performed as described previously (Yamaoka *et al.*, 1998b). For histology, all biopsy specimens were fixed in 10 % buffered formalin for 24 h and then embedded in paraffin. Serial sections were stained with haematoxylin–eosin and May–Giemsa. The degree of bacterial load was classified into four grades according to the updated Sydney System (Dixon *et al.*, 1996). Grade $\geqslant 1$ bacterial load was defined as *H. pylori*-positive. Patients were considered *H. pylori*-negative when all four tests were negative, whereas the *H. pylori*-positive status required at least one positive result.

IHC. IHC was performed as described previously (Uchida *et al.*, 2007). Briefly, after antigen retrieval and inactivation of endogenous peroxidase activity, tissue sections were incubated with anti-*H. pylori* antibody (Dako) overnight at 4 °C. After washing, the sections were incubated with biotinylated goat anti-rabbit IgG (Nichirei), followed by incubation with a solution of avidin-conjugated horseradish peroxidase (Vector). In all cases, we performed Giemsa staining using a serial section to identify the presence of *H. pylori*. If the *H. pylori* identified by Giemsa staining was positively immunostained, we considered the patient to be *H. pylori*-positive.

Staging for gastritis. The degree of gastritis was classified using four grades according to the updated Sydney System (Dixon *et al.*, 1996); samples of grade $\geqslant 1$ were considered atrophy-positive, according to a previous report (Bornschein *et al.*, 2012). In addition, the gastritis stage was assessed based on the severity and topographic locations (antrum and corpus) according to the Operative Link on Gastritis Assessment (OLGA) system (Rugge *et al.*, 2005, 2007).

Isolation and genotyping of *H. pylori*. *H. pylori* DNA was extracted from *H. pylori* cultured to confluence on plates using a commercially available kit (Qiagen). The status of *cagA* was determined with PCR for a conserved region of *cagA* and for direct sequencing as described previously (Yamaoka *et al.*, 2000). The *cagA* genotype (East-Asian type and Western type) was confirmed by sequencing the PCR products as described previously (Xia *et al.*, 2009). We performed *vacA* genotyping (s1, s2, m1 and m2) as described previously (Atherton *et al.*, 1995; Yamaoka *et al.*, 1999a). The PCR products were analysed by gel electrophoresis using 1.5 % agarose gel containing ethidium bromide.

Statistical analysis. Data were analysed using SPSS version 19 (SPSS). Statistical evaluation was performed with the χ^2 test to compare discrete variables, and with the Mann–Whitney U test and the t-test to compare continuous variables. To match age and sex, we used multiple backward stepwise logistic regression analyses to examine the associations of peptic ulcer with the main predictor variables. Predictor variables for peptic ulcer consisted of age, sex and *H. pylori* status. For each variable, the odds ratio and 95 % confidence interval were calculated. A two-tailed $P<0.05$ was considered significant.

RESULTS

Prevalence of *H. pylori* infection in the Dominican Republic

Table 1 shows the *H. pylori*-positive rate for each test. The histology and IHC results matched completely, and are

Table 1. Prevalence [*n* (%)] of *H. pylori* infection determined by diagnostic tests

	Age (years)					Total
	17–29	30–39	40–49	50–59	60-91	
Total no. subjects	24	28	40	30	36	158
Rapid urease test	13 (54.2)	14 (50.0)	22 (55.0)	16 (53.3)	13 (36.1)	78 (49.4)
Culture	10 (41.7)	14 (50.0)	21 (52.5)	12 (40.0)	11 (30.6)	68 (43.0)
Histological examination	14 (58.3)	17 (60.7)	24 (60.0)	16 (53.3)	18 (50.0)	89 (56.3)
Final	15 (62.5)	17 (60.7)	25 (62.5)	18 (60.0)	18 (50.0)	93 (58.9)

shown as 'histological examination'. Compared with the positive rate of the culture, that of histological examination was significantly higher (P=0.01). Although not statistically significant, the rapid urease test showed a positive rate lower than that of the histological examination. Overall, the prevalence of *H. pylori* infection in the Dominican Republic was 58.9 % (93/158). Fig. 1 shows the prevalence of *H. pylori* infection in various age groups. No relationship was found between *H. pylori* infection rate and age ranging from 17 to 91 years (P=0.81).

Endoscopic findings and *H. pylori* infection rate

Gastritis was the most common finding (134/158, 84.8 %). Among 134 patients with gastritis, four had gastric polyps and one had a submucosal tumour. Gastric and duodenal ulcers were found in 13 (8.2 %) and 10 (6.3 %) patients, respectively. Gastric cancer was found in one patient (0.6 %). Fig. 2 shows the prevalence of *H. pylori* infection in patients with gastritis and peptic ulcer. A high infection rate was detected among patients with gastric ulcer (84.6 %) and duodenal ulcer (80.0 %), whereas 52.8 % of the patients with gastritis were *H. pylori*-positive. When gastric and duodenal ulcers were defined as peptic ulcers, the prevalence of *H. pylori* infection in peptic ulcer was significantly higher than that in gastritis (82.6 versus 54.5 %, P=0.008). Multiple logistic analysis after adjustment for age and gender showed that *H. pylori* positivity was significantly associated with peptic ulcer (odds ratio 3.96; 95 % confidence interval 1.28–12.29). The patient with gastric cancer was infected with *H. pylori*.

Virulence genes of *H. pylori*

DNA was successfully extracted from 64 of 68 cultured strains and virulence genes were examined in these 64 strains (47 from patients with gastritis, 16 from patients with peptic ulcer and one from the patient with gastric cancer). The distributions of the *cagA* and *vacA* genotypes in the Dominican Republic are shown in Table 2. The prevalence of *cagA* was 75.0 % (48/64). The *vacA* s1 genotype was the most common (48/64, 75.0 %). The prevalence of the *vacA* m1 genotype was 71.9 % (46/64). Among the genotypes combining the *vacA* s and m regions, 44 (68.8 %) were s1m1, four (6.3 %) were s1m2, two (3.1 %) were s2m1 and 14 (21.9 %) were s2m2. Among the genotypes combining *cagA* and *vacA*, the *cagA*-positive/*vacA* s1m1 genotype was the most prevalent (43/64, 67.2 %). The types of *cagA* and *vacA* were compared between patients with gastritis and peptic ulcer (Table 2). No differences in the

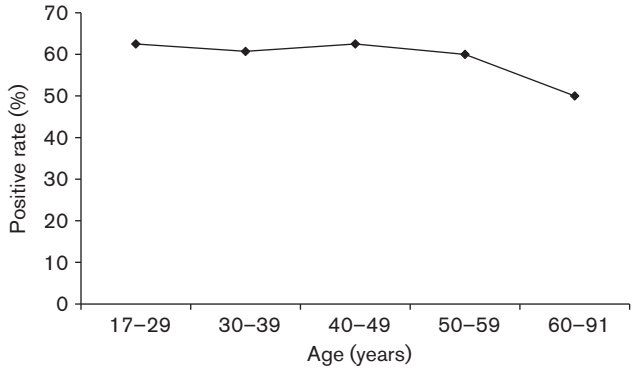

Fig. 1. Prevalence of *H. pylori* infection in various age groups in the Dominican Republic. *H. pylori* infection was examined using four methods: rapid urease test, culture test, histological test and IHC. Patients were considered *H. pylori*-negative when all four tests were negative, whereas the *H. pylori*-positive status required at least one positive test result.

Fig. 2. Prevalence of *H. pylori* infection in patients with gastritis and peptic ulcer.

Table 2. Virulence genes [*n* (%)] of *H. pylori* in the Dominican Republic

	Total	Gastritis	Peptic ulcer	Gastric cancer
N	64	47	16	1
cagA (+)	48 (75.0 %)	36 (76.6)	12 (75.0)	0 (0.0)
cagA (–)	16 (25.0)	11 (23.4)	4 (25.0)	1 (100.0)
vacA s1	48 (75.0)	36 (76.6)	12 (75.0)	0 (0.0)
vacA s2	16 (25.0)	11 (23.4)	4 (25.0)	1 (100.0)
vacA m1	46 (71.9)	34 (72.3)	12 (75.0)	0 (0.0)
vacA m2	18 (28.1)	13 (27.7)	4 (25.0)	1 (100.0)
vacA s1m1	44 (68.8)	33 (70.2)	11 (68.8)	0 (0.0)
vacA s1m2	4 (6.3)	3 (6.4)	1 (6.3)	0 (0.0)
vacA s2m1	2 (3.1)	1 (2.1)	1 (6.3)	0 (0.0)
vacA s2m2	14 (21.9)	10 (21.3)	3 (18.8)	1 (100.0)
cagA (+) *vacA* s1m1	43 (67.2)	32 (68.1)	11 (68.8)	0 (0.0)
cagA (+) *vacA* s1m2	3 (4.7)	2 (4.3)	1 (6.3)	0 (0.0)
cagA (+) *vacA* s2m1	1 (1.6)	1 (2.1)	0 (0.0)	0 (0.0)
cagA (+) *vacA* s2m2	1 (1.6)	1 (2.1)	0 (0.0)	0 (0.0)
cagA (–) *vacA* s1m1	1 (1.6)	1 (2.1)	0 (0.0)	0 (0.0)
cagA (–) *vacA* s1m2	1 (1.6)	1 (2.1)	0 (0.0)	0 (0.0)
cagA (–) *vacA* s2m1	1 (1.6)	0 (0.0)	1 (6.3)	0 (0.0)
cagA (–) *vacA* s2m2	13 (20.3)	9 (19.1)	3 (18.8)	1 (100.0)

cagA and *vacA* genotypes were found between the gastritis and peptic ulcer groups (all $P>0.05$).

The CagA types derived from the *cagA* genotypes by gene sequencing were also examined. Thirty-nine strains were sequenced successfully for *cagA*. All 39 showed Western-type CagA. Among the 39 Western-type CagA strains, 33 (84.6 %) were ABC type. Strains with multiple C segments (i.e. ABCC) were found in three patients (two with gastritis and one with gastric ulcer). Other types of CagA (AB, AABC and ABBC) were found in one patient each.

Table 3. Differences in histological scores according to the status of *H. pylori* infection

	H. pylori (+)	*H. pylori* (–)	*P*
N	93	65	
Antrum			
Activity	1.33±0.64	0.12±0.37	<0.0001
Inflammation	1.81±0.63	0.51±0.71	<0.0001
Atrophy	1.04±0.53	0.66±0.53	<0.0001
Intestinal metaplasia	0.14±0.52	0.08±0.40	0.33
Bacterial density	1.58±0.82	0.00±0.00	<0.0001
Corpus			
Activity	1.00±0.67	0.11±0.40	<0.0001
Inflammation	1.26±0.56	0.32±0.58	<0.0001
Atrophy	0.40±0.55	0.12±0.37	<0.0001
Intestinal metaplasia	0.10±0.44	0.05±0.27	0.48
Bacterial density	1.60±0.75	0.00±0.00	<0.0001
OLGA score	1.08±0.55	0.68±0.56	<0.0001

Gastric mucosa status

Histological findings classified 35 patients as grade 0 for atrophy in the antrum; 106 patients had grade 1, 17 had grade 2 and none had grade 3. Atrophy in the corpus was classified as grades 0, 1 and 2 for 117, 37 and four patients, respectively. When samples of grade $\geqslant 1$ were considered atrophy-positive, 123 patients (77.8 %) were found to have mucosal atrophy in the antrum, and 41 (25.9 %) patients also had mucosal atrophy in both the antrum and the corpus.

The staging of gastritis was also assessed according to the OLGA system. Stage 0 was found in 22.1 %, stage I in 64.5 % (102/158) and stage II in 13.2 % (21/158). Stages III and IV were not found. The differences in histological scores according to the status of *H. pylori* infection are shown in Table 3. Compared with *H. pylori*-negative subjects, *H. pylori*-positive subjects showed significantly greater activity, inflammation and atrophy in both the antrum and the corpus (all $P<0.0001$). The OLGA score in *H. pylori*-positive subjects was also significantly higher than that in negative patients (1.08±0.55 versus 0.68±0.56, $P<0.0001$). Eleven and seven subjects had intestinal metaplasia in the antrum and corpus, respectively. The prevalence of intestinal metaplasia in the antrum was not significantly different between *H. pylori*-positive and *H. pylori*-negative subjects (8.6 versus 6.1 %, $P=0.56$). Likewise, no differences in the presence of intestinal metaplasia in the corpus were found between *H. pylori*-positive and *H. pylori*-negative subjects (5.3 versus 3.0 %, $P=0.48$).

Histological scores according to *cagA* status are shown in Table 4. Compared with *cagA*-negative patients, *cagA*-positive patients had significantly higher scores for atrophy

Table 4. Histological scores according to the status of *cagA*

	cagA (+)	*cagA* (–)	*P*
N	48	16	
Antrum			
Activity	1.48±0.61	1.25±0.44	0.20
Inflammation	1.94±0.56	1.75±0.57	0.24
Atrophy	1.15±0.50	0.81±0.54	0.03
Intestinal metaplasia	0.13±0.53	0.00±0.00	0.31
Bacterial density	1.79±0.68	1.63±0.80	0.48
Corpus			
Activity	1.06±0.63	0.81±0.65	0.17
Inflammation	1.31±0.55	0.94±0.57	0.03
Atrophy	0.42±0.49	0.06±0.25	0.01
Intestinal metaplasia	0.06±0.43	0.00±0.00	0.56
Bacterial density	1.81±0.67	1.38±0.71	0.03
OLGA score	1.15±0.50	0.81±0.54	0.03

in the antrum and corpus (P=0.03 and 0.01, respectively). Inflammation scores in the corpus of *cagA*-positive patients were also significantly higher than in those of *cagA*-negative patients (P=0.03). Interestingly, none of the 16 *cagA*-negative patients had intestinal metaplasia in the antrum or corpus. The OLGA score in *cagA*-positive patients was also significantly higher than that in *cagA*-negative patients (1.15±0.50 versus 0.81±0.54, P=0.03).

DISCUSSION

Using a combination of four tests, we determined the prevalence of *H. pylori* in the Dominican Republic to be 58.9 %. Similar results have been reported in neighbouring countries, such as Haiti (60.0 %) and Jamaica (50.8 %) (Hisada *et al.*, 2001; Shak *et al.*, 2011). In contrast with those in developed countries, *H. pylori* infections in the developing world occur earlier in life and with a higher frequency (Goh *et al.*, 2011). The prevalence of *H. pylori* infection was reported to be >70 % in developing countries (Calvet *et al.*, 2013; Leja *et al.*, 2012; Porras *et al.*, 2013). The present study showed that the prevalence was high even in young age groups (58.3 % in patients aged ⩽29 years) in the Dominican Republic. The high infection rate in the younger age group in our study was consistent with that reported in a study conducted in 2001–2002 (Aoki *et al.*, 2005). Sanitary conditions (e.g. availability of necessary equipment for water and sewage management) are considered important factors in *H. pylori* infection (Goh *et al.*, 2011). Poor sanitary conditions are positively associated with a high rate of *H. pylori* positivity (Dattoli *et al.*, 2010; Strebel *et al.*, 2010). Furthermore, the prevalence of *H. pylori* infection decreased along with improvements in clean public water systems after World War II in Japan (Asaka *et al.*, 1992). The World Health Organization and UNICEF have reported that the percentage of improved sanitation facilities in 2011 was 82 % in the Dominican Republic. By contrast, that figure was 100 % in Japan and the United States, where *H. pylori* infection rates are decreasing (http://www.unicef.org/). Unimproved sanitary conditions in the Dominican Republic may be associated with high *H. pylori* infection rates. Nevertheless, the prevalence of *H. pylori* infection was lowest in the older age group and we reported previously that the prevalence of *H. pylori* infection decreased with age in Bhutan, which is also a developing country (Vilaichone *et al.*, 2013). The decrease in *H. pylori* infection rate with age might be due to frequent antibiotic use in infectious diseases in general, but further studies are necessary to clarify the factors influencing the *H. pylori* infection rate.

Our finding that the prevalence of *H. pylori* in patients with peptic ulcer was significantly higher than that in patients with gastritis was consistent with the findings of previous studies (Huang *et al.*, 2002; Malfertheiner *et al.*, 2012; Papatheodoridis *et al.*, 2006). This relationship suggests that *H. pylori* infection is a risk factor for the development of peptic ulcer and gastric cancer in the Dominican Republic. Furthermore, histological scores were higher in *H. pylori*-positive patients than in *H. pylori*-negative patients, consistent with the results of other studies (Kodama *et al.*, 2012b). Successful eradication is effective for the prevention of not only peptic ulcer, but also gastric cancer (Asaka *et al.*, 2010). Additionally, 10-year prospective follow-up studies have shown that successful eradication therapy reduces significantly mucosal inflammation, activity and atrophy (Kodama *et al.*, 2012a, b). Therefore, eradication therapy for *H. pylori* infection can be useful in reducing the occurrence of peptic ulcer and gastric cancer in the Dominican Republic.

We found that 77.8 % of subjects had mucosal atrophy in the antrum, and 25.9 % subjects also had mucosal atrophy in both the antrum and the corpus in the Dominican Republic. We reported previously that mucosal atrophy was found in the antrum in 91.9 % of subjects and in the corpus in 37.7 % of subjects in Bhutan, where the incidence of gastric cancer is high (17.2 : 100 000 year^{-1}) (Shiota *et al.*, 2013). Another staging system for gastritis (OLGA) showed that 22.1 % of subjects were classified as stage 0, 64.5 % as stage I and 13.2 % as stage II in the Dominican Republic. Stages III and IV were not observed. On the contrary, ~40 % of subjects were classified as stages III and IV in a study of Japanese patients (Satoh *et al.*, 2008). Milder gastritis might be related to the low incidence of gastric cancer in the Dominican Republic despite the high *H. pylori* infection rate.

To our knowledge, this report is the first to reveal the virulence factors of *H. pylori* in the Dominican Republic. The *cagA* gene, which encodes a highly immunogenic protein (CagA), is the most extensively studied *H. pylori* virulence factor (Covacci *et al.*, 1993; Tummuru *et al.*, 1993). Almost all *H. pylori* isolates from East-Asian countries and ~60–80 % of isolates from Western countries

are *cagA* positive (Matsuda *et al.*, 2011; Suzuki *et al.*, 2012; Yamaoka *et al.*, 1999b). In Western countries, individuals infected with *cagA*-positive *H. pylori* strains reportedly have a higher risk of peptic ulcer or gastric cancer than those infected with *cagA*-negative strains (van Doorn *et al.*, 1998; Yamaoka *et al.*, 2002). Furthermore, CagA can be divided into two types (East-Asian and Western) according to the repeat sequences of the 3′ region of *cagA* (Yamaoka, 2010). The repeat regions contain the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs. Sequences have been annotated according to segments (20–50 aa) flanking the EPIYA motifs (i.e. segments EPIYA-A, -B, -C and -D) (Yamaoka, 2010). Compared with individuals with Western-type CagA strains containing EPIYA-C segments, those infected with East-Asian-type CagA strains containing EPIYA-D segments reportedly have an increased risk of peptic ulcer or gastric cancer (Matsunari *et al.*, 2012; Vilaichone *et al.*, 2004). We determined the prevalence of *cagA* to be 75.0 % in the Dominican Republic, which is similar to that of neighbouring countries. The *cagA*-positive rate is reportedly 73.2 % in Cuban strains (Torres *et al.*, 2009). Furthermore, all of the 39 strains sequenced successfully were Western-type CagA; in particular, 84.6 % of CagA strains showed the ABC pattern. The prevalence of strains with multiple C segments was only 7.7 % and all of them were ABCC, but not ABCCC. The percentage of strains with ABCC was lower than that in Cuban strains (20 %) (Torres *et al.*, 2012). In Western countries, the incidence of gastric cancer in patients infected with strains carrying multiple EPIYA-C repeats is higher than the incidence in those infected with strains with a single repeat (Argent *et al.*, 2004; Azuma *et al.*, 2002; Xia *et al.*, 2009; Yamaoka *et al.*, 1998a, 1999a). For example, 31 % of *H. pylori* strains had multiple EPIYA-C repeats in Colombia, where the incidence of gastric cancer is relatively high (17.4 : 100 000 year^{-1}) (Yamaoka *et al.*, 1999a). Western-type CagA and the lower percentage of multiple EPIYA-C repeats in the Dominican Republic may account for the lower incidence of gastric cancer in the Dominican Republic than in East-Asian countries. However, we found that *cagA*-positive patients had significantly greater atrophy in the antrum and corpus than *cagA*-negative patients. Inflammation score in the corpus was also significantly higher in these patients. Interestingly, none of 16 *cagA*-negative patients had intestinal metaplasia in the antrum or corpus. These results suggest that patients infected with *cagA*-positive *H. pylori* strains can be a high-risk population, even in the Dominican Republic.

Many studies in Western countries have shown that individuals infected with *vacA* s1 or m1 *H. pylori* strains have a higher risk of peptic ulcer or gastric cancer than those infected with s2 or m2 strains (Atherton *et al.*, 1995; Cover & Blaser, 1992; Sugimoto & Yamaoka, 2009; Sugimoto *et al.*, 2009). The *vacA* s1m1 genotype was predominant in the Dominican Republic, as in Jamaica and Cuba (Hisada *et al.*, 2001; Torres *et al.*, 2009). On the contrary, 21.9 % of subjects had the *vacA* s2m2 genotype – a prevalence higher than that in Japan, where most cases are *vacA* s1m1 (Yamaoka *et al.*, 1998b). Furthermore, *vacA* s2m1 or *vacA* s2m2 genotypes were found even in *cagA*-positive strains and this is consistent with findings in Cuban strains (Torres *et al.*, 2009). Less virulent types of *vacA* may also be related to the lower incidence of gastric cancer in Caribbean countries including the Dominican Republic.

However, our study had several limitations. The prevalence of *H. pylori* infection in this study was determined based on a combination of four analyses. Although several clinical tests have been developed to diagnose *H. pylori* infection, no 'gold standard' has been established. In this study, histological examination showed the highest positive rate. The histological diagnosis of *H. pylori* infection is very much dependent on the expertise of the pathologists. Rapid urease tests, such as CLOtest, can be useful for rapid diagnosis. However, the accuracy of this test can be affected by the bacterial load (Megraud & Lehours, 2007) and a reportedly low sensitivity (Megraud, 1997). The results of cultures from biopsy specimens are dependent on transport conditions and careful handling in the laboratory (Megraud & Lehours, 2007). Other global tests, such as the urea breath test or the stool *H. pylori* antigen test, should be used in future studies.

In conclusion, we found that the prevalence of *H. pylori* infection in the Dominican Republic was high despite the low incidence of gastric cancer. Lower virulence of *H. pylori* and mild gastritis in the Dominican Republic might contribute to the low incidence of gastric cancer. However, the presence of *H. pylori* was related to severe clinical outcomes and more severe gastritis. In addition, the presence of *cagA* was related to more severe gastritis. Therefore, eradication therapy for *H. pylori* would likely contribute to decreasing *H. pylori*-related diseases such as peptic ulcer and gastric cancer in the Dominicaln Republic.

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