

Original article:

Pathogenic potential of *Helicobacter pylori* strains can explain differences in *H. pylori* associated diseases rates from Chile and Cuba.

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Abstract:

Background: The prevalence of *Helicobacter pylori*-related diseases varies geographically and it is partially determined by the virulence of the circulating strains. Cuba and Chile exhibit different gastric cancer rates, on despite of very similar *H. pylori* infection rates. We determined if differences in the pathogenic potential of *H. pylori* isolates from Chile and Cuba could explain the disease outcome in each population. **Methods:** *H. pylori* isolates from 78 Chilean and 71 Cuban patients were analyzed using PCR for the presence of *cagA*, *babA2*, *vacA* alleles and the pattern of EPIYA motifs. **Results:** *cagA* was detected in 94.9 % of Chilean and 64.7 % of Cuban isolates ($P < 0.001$) and was significantly associated with duodenal ulcer (DU) in Cuba ($P < 0.01$) but not in Chile. The presence of *cagA* with multiple EPIYA-C motifs was 18.2 % higher in Chile than in Cuba ($P < 0.05$). Also, an association was observed between GU ($P \leq 0.05$) and premalignant lesions ($P < 0.001$) with the multiple EPIYA-C motif status of the strains in Chile, but not in Cuba. The prevalence of *vacA s2m2* genotype was predominant in Chile (66.7 %), while in Cuba was prevalent the *s1m1* genotype (56.8 %); and the last one was significantly associated with the presence of DU in Cuban patients. **Conclusions:** The *cagA* status and the EPIYA pattern found in Chilean and Cuban *H. pylori* clinical isolates partially explain the differences in disease prevalence between both countries. The high proportion of *vacA s2m2* genotype in Chile was an unexpected result, needing further studies.

Keywords: *Helicobacter pylori*, EPIYA motifs, Gastric cancer, Virulence

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Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative spiral-shaped bacterium that colonizes the human stomach. In this sense, colonization constitutes an established risk factor in the pathogenesis of

functional dyspepsia, peptic ulceration, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma^{1, 2}. The prevalence of these pathologies varies considerably throughout the world. Among the multiple factors responsible of this

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dissimilar prevalence, *H. pylori* strain heterogeneity seems to be a crucial factor^{1,3,4}.

Although many virulence factors have been reported to *H. pylori*, only few of them have been linked with disease, and particularly with gastric cancer⁴.

CagA protein is the most recognized *H. pylori* virulence marker^{3, 5}. The *cagA*-positive strains are associated with increased risk of peptic ulceration, atrophic gastritis and gastric adenocarcinoma⁶. The CagA protein is injected into the eukaryotic cells where is phosphorylated and binds to SHP-2 phosphatase. CagA-SHP-2 complexes disrupt signal transduction pathways developing cell atrophy^{5, 6}. Phosphorylation of CagA occurs in the C-terminal region within the EPIYA motifs, which are classified as A, B, C and D⁷. The EPIYA-A and B motifs appear in almost all Western and East Asian *H. pylori* strains while EPIYA-C, in a single or multiple repeat, appears in Western isolates and EPIYA-D is restricted to Eastern strains. The EPIYA-D motif exhibits the greatest levels of CagA phosphorylation and SHP-2 binding follow by strains carrying multiple EPIYA-C motifs^{8, 9}. Consequently, both genotypes have been associated with higher GC rates in several studies^{9, 10, 11}. Therefore, detection of the EPIYA patterns has become an important tool to determine the carcinogenic potential of *H. pylori* isolates.

Another major *H. pylori* virulence marker is the vacuolating cytotoxin (VacA), which causes induction of cytoplasmic vacuoles, mitochondrial damage and inhibition of T cell activation^{3,12}. Polymorphisms among *vacA* gene regions result in different levels of vacuolation and damage. The major variations are presented within signal (*s1* and *s2*), intermediate (*i1* and *i2*) and middle (*m1* and *m2*) regions. It has been determined that *s* region is related with vacuolation while the *m* region determines the cell specificity^{13, 14}. In general, the *vacA s1m1* genotype exhibits the highest vacuolation activity, *s1m2* a moderate activity and *s2m2* and *s2m1* combinations are non-toxic¹⁴. The *vacA s1* and *m1* alleles have been associated with *H. pylori*-related diseases, including GC^{15, 16, 17}. Finally, the other well-established virulence marker is the blood group binding antigen (BabA)³. The attachment of *H. pylori* to gastric epithelial cells facilitates colonization and delivery of virulence factors such as CagA and VacA into eukaryotic cells^{12,17}. BabA is encoded by the *babA2* gene and is important for *H. pylori* adherence. There are two distinct *babA* alleles (*babA1* and *babA2*) and one highly homologous gene, *babB*, but only *babA2* is functionally active. The sequence of *babA1*

and *babA2* only differed in a 10 bp deletion in the signal peptide sequence of *babA1* that eliminates its translational initiation codon¹⁸. Several studies have suggested an association between *babA2*-positive strains and increased risk of developing severe gastric diseases in Western countries^{19, 20}; however this association does not appear in others studies²¹. Nevertheless, the detection of this virulence factor remained important to characterize pathogenicity of *H. pylori* strains.

Gastric cancer is a world health burden, ranging as the second cause of cancer death worldwide²². However, the rates of incidence and mortality differ markedly from one region to another all over the world^{22, 23}, even when *H. pylori* infection prevalence is similar^{24,25}. That is the case for the different mortality rates reported for Chile and Cuba; the former is 14/100 000²⁶ and the latter 7.5/100 000²⁷, exhibiting similar estimated *H. pylori* infection rates, of 74.5 and 73 %, respectively^{28,29}.

We hypothesized that this difference may be mainly attributed to differences in the pathogenic potential of circulating *H. pylori* strains. Thus, this study aimed to compare the prevalence of *H. pylori* virulence factors in two specific populations, from two Latin American countries, and their association with *H. pylori*-association diseases finding

Materials and Methods:

Patients and samples

Gastric biopsy samples were obtained from 200 consecutively *H. pylori*-infected patients of both genders that undergoing routine upper gastrointestinal endoscopy at Medical and Surgical Research Hospitals, Havana city, Cuba and Regional Hospital of Talca, Maule, Chile. The mean age of the one hundred Cuban patients was 49.6 (range, 18 to 79) years and that of the one hundred Chilean patients was 52.0 (range, 18 to 82). All Chilean and Cuban patients were from the Region del Maule and from The Havana province, respectively. We included subjects greater than 18 years old with dyspeptic symptoms. Patients with had received previous treatment for *H. pylori* infection, antibiotics, acid-reducing drugs, such as H2-receptor antagonists, acid pump inhibitors, nonsteroidal anti-inflammatory drugs, or bismuth compounds in the last four weeks were excluded.

Endoscopic observation and histological confirmations were used to determine patient pathologies as duodenal ulcer (DU), gastric ulcer (GU), gastritis (G) and premalignant lesion (PL). Peptic ulcer and gastric cancer were identified using

endoscopy. Gastritis was diagnosed in the absence of peptic ulcer or gastric malignancy.

Experienced endoscopists of each country collected four gastric biopsy specimens during endoscopy session: three two samples from the antrum, approximately 2–3 cm from the pyloric ring, and one sample from the greater curvature of 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.

The biopsies culture for *H. pylori* isolation were made in each country, and the strains isolated in Cuba were translated to Catholic University of Maule for DNA isolation and PCR analysis. The biopsy materials were fixed in 10% buffered formalin for 24 h and then embedded in paraffin in each country. The paraffin block from both countries were stained and examined in a blind test by the same experienced pathologist at Regional Hospital of Talca, Maule, Chile in order to minimize potential bias.

Histology

Gastric biopsy specimens for histopathology were stained using hematoxylin and eosin (stain for the detection of *H. pylori*), and the histological analysis of the gastric mucosa was performed according to the Updated Sydney System³⁰. In addition, on the basis of the topographic locations (antrum and corpus), the gastritis stage (the severity and topography of atrophy) was assessed according to the Operative Link on Gastritis Assessment (OLGA) system³¹.

***H. pylori* Culture and Genotyping**

Antral biopsy specimens were obtained for the isolation of *H. pylori* using standard culture methods as previously described^{18, 29}. *H. pylori* identified based on their typical morphology, gram staining, and positive reaction for urease, oxidase and catalase test.

The reference strains J99 and CCUG17874, were kindly provided by Professor Francis Mégraud from Pellegrin Hospital, Bourdeaux, France and Professor Ann-Mary Svennerholma from Gothenburg University, Sweden.

Genomic DNA was extracted and purified from each *H. pylori* isolate. PCR was used to detect the *babA*, *cagA*, *vacA* genes and the *cagA* EPIYA motifs. Primer pairs for *babA*, *cagA* and *vacA* are presented in Table I. The number and type of EPIYA motifs were determined as previously reported [32]. PCR were performed in a 25 μ L reaction containing 1.25 U Taq polymerase (Roche, Germany), 50 ng of genomic DNA and 0.6 mM of each primer. PCR products were

visualized by agarose gel electrophoresis. Reference strains with previously characterized genotypes were used as positive controls.

Data analysis

Variables such as gender, age and the presence of each candidate gene were evaluated. The univariate association between each genotype and the clinical presentations were quantified by the Chi-square (χ^2) or Fisher's exact (FE) tests. Independent samples t and one way ANOVA tests were used to compare quantity variables. A *P* value of less than 0.05 was accepted as statistically significant. The SPSS statistical software package version 16.0 was used for all statistical analyses. Mixed *vacA* genotypes were excluded from comparisons.

Ethical clearance: The study protocol was approved by the ethics and research committees of Cuban a Chilean Hospitals, The National Centre for Scientific Research, Havana, Cuba and Catholic University of Maule, Chile. All patients gave an informed consent for endoscopy and participation in the study.

Results

In the present study, 71 Cuban and 78 Chilean patients were *H. pylori* positive. From endoscopic observations: 53.5 % of Cuban and 62 % of Chilean patients were diagnosed with gastritis (G), 14.1 % of Cuban and 38 % of Chilean patients had gastric ulcer (GU) and 32.4 % of Cuban patient had duodenal ulcer (DU). Histological analysis revealed intestinal metaplasia and dysplasia in 39.8 % and 4 % of Chilean patients, respectively. Statistical differences between these populations were found for the appearance of GU, DU and premalignant lesions, in all cases with $P < 0.001$.

Prevalence of *cagA*-positive strains among Cuban and Chilean populations

The *cagA* gene was present in 64.7% (46/71) and 94.9% (74/78) of the *H. pylori* strains from Cuba and Chile ($P < 0.001$), respectively (Table II). Furthermore, a significant association was found between *cagA* status and DU ($P < 0.01$) for Cuban patients. No association was found between the presence of *cagA* and any pathology in the Chilean group, due to the high *cagA* positivity in these isolates.

***CagA* phosphorylation motifs**

All the *cagA*-positive isolates were evaluated to determine their EPIYA motifs pattern. We turned our attention mainly in the number of EPIYA-C motifs. Among Cuban isolates, the number of EPIYA-C motifs ranged from 0 to 3; there were 33 with 1 (71.7 %), 12 with 2 (26.1 %) and 2 with 3 (4.3 %), and thus

Table I. Primer pairs used for PCR genotyping of *Helicobacter pylori* isolates

Genes	Primer sequence (5'-3')	PCR product (bp)	References
<i>glmM</i>			
glmMF	CCCTCACGCCATCAGTCCCAAAAA	417	[33]
glmMR	AAGAAGTCAAAAACGCCCAAAAC		
<i>cagA</i>			
cagF1	GATAACAGGCAAGCTTTTGA	349	[34]
cagB1	CTGCAAAAGATTGTTTGGCAGA		
EPIYA motifs			
cag2	GGAACCCTAGTCGGTAATG	-	[35]
cag4	ATCTTTGAGCTTGTCTATCG	Variable (400-750)	
cagA-P1C	GTCCTGCTTTCTTTTTATTAACCTTAGC	174	
cagA-P2 CG	TTTAGCAACTTGAGCGTAAATGGG	222	
cagA-P3E	ATCAATTGTAGCGTAAATGGG	378	
cagEpi-C	AAAGGTCCGCCGAGATCA	397	
cagEpi-D	GCCTGCTTGATTGCCCTCATC	399	[32]
cagAEast ^a	AGAGGGAAGCCTGCTTGATT	495	[11]
<i>babA</i>			
bab7-F	CCAAACGAAACA AAAAGC GT	271	[36]
bab7-R	GCTTGTGTA AAAAGCCGTCGT		
babA2F	AATCCAAAAGGAGAAAAAGTATGAAA	832	[32]
babA2R	TGTTAGTGATTTCCGGTGTAGGACA		
babA2R607 ^b	GTTTTCTTTGAGCGCGGGTAAGC	607	[37]
<i>vacA</i>			
vacAsF	ATGGAAATACAACAACACAC	s1-259/s2-286	[13]
vacAsR	CTGCTTGAATGCGCCAAAC		
vacAmF	CAATCTGTCCAATCAAGCGAG	m1-567/m2-642	
vacAmR	GCGTCAAAAATAATTCCAAGG		

^a Reverse primer cagAEast was used to corroborate the presence of EPIYA-D genotype; due to our primer cagEpi-D has not been extensively used.

^b Primer designed by Zambon et al²⁸ and modified by Lino et al²⁷.

30.4 % (14/46) of *cagA*-positive strains harbored 2 or more EPIYA-C motifs. In the case of Chile, the number of EPIYA-C motifs ranged also from 0 to 3; 34 with 1 (35.4 %), 26 with 2 (35.1 %) and 10 with 3 (13.5 %), and thus 48.6 % (36/74) carried 2 or more EPIYA-C motifs. This was a higher proportion than the founded in Cuban isolates ($P < 0.05$).

No association was found between the presence of more than two EPIYA-C motifs and any pathology present in Cuban patients. On the contrary, it

was found a correlation between this status and GU ($P \leq 0.05$) and a more strong association with premalignant lesions ($P < 0.001$), which included dysplasia and intestinal metaplasia, in the Chilean group (Table III).

***vacA* polymorphism**

Among the Chilean isolates, 4/78 (5.1 %) possessed both *s* types and 5/78 (6.8 %) possessed both *m* types. These mixed genotypes were not seen in Cuban isolates. The most virulent *sI* allele was

predominantly present in Cuban *H. pylori* isolates with 73.2 %, whereas 26.8 % of isolates had the *s2* genotype. On the contrary, Chilean strains showed a prevalence of 19.2 % for *s1* type and 75.6 % for *s2* genotype. The same inverted proportions were observed for middle-region genotypes; in Cuban strains 56.3 % and 43.7 % presented *m1* and *m2* genotypes, respectively, whereas 11.5 % and 76.9 % of Chilean isolates had the *vacA m1* and *m2* alleles. The *vacA s* alleles without either, *m1* or *m2* allele were detected in 1.4 % and 5.1 % of Cuban and Chilean isolates, respectively. Additionally, the prevalence of *s* and *m* allele combinations was as follow: the *s1m1* genotype was predominant (56.8 %) in Cuba, but in

Chile it was the *s2m2* genotype (66.7%) (Table II). Significant correlation was only found between the presence of *vacA s1* and *vacA s1m1* genotypes and DU ($P < 0.05$) in Cuba (Table II), due to the high prevalence of *s2*, *m2* and *s2m2* genotypes found in Chilean isolates.

***babA2* genotype**

In both countries, the presence of *babA2*-positive strains was high (Table II); in Chile 97.4 % (76/78) of the strains carried this allele, while 88.7 % (63/71) of Cuban isolates were *babA2* positive ($P < 0.05$). No significant association was found for the presence of *babA2* gene and any pathology observed in both populations (Table III).

Table II. Characterization of virulence factors genotypes in the Cuban and Chilean *H. pylori* isolate

	Chile n= 69 ^a						Cuba n= 71			
	<i>s1m1</i>	<i>s1m2</i>	<i>s2m2</i>	<i>s2m1^b</i>	<i>s2m^{-b}</i>	<i>s1m⁻</i>	<i>s1m1</i>	<i>s1m2</i>	<i>s2m2</i>	<i>s1m⁻</i>
<i>cagA</i> +	4	10	43	5	3	1	36	7	2	1
<i>cagA</i> -	ND	ND	3	ND	ND	ND	4	4	17	ND
<i>babA2</i> +	4	10	44	5	3	1	39	10	13	1
<i>babA2</i> -	ND	ND	2	ND	ND	ND	1	1	6	ND
Total (%)	4 (5.8)	10 (14.9)	46 (66.7)	5 (7.5)	3 (4.3)	1 (1.4)	40 (56.3)	11 (15.5)	19 (26.8)	1 (1.4)

ND, not detected.

^a Nine Chilean isolates were not analyzed due to the presence of mixed genotypes.

^b These genotypes were not detected in any Cuban isolate.

Table III. Distribution of virulence factors between diseases found in Chile and Cuba.

Genotypes	Pathologies					
	Chile			Cuba		
	G ^a	GU ^b	PL	G ^a	GU	DU
	n= 31 (%)	n= 30 (%)	n= 34 (%)	n=38 (%)	n= 10 (%)	n= 23 (%)
<i>vacA s1m1</i>	1 (3.2)	ND	3 (8.8)	21 (55.3)	2 (20)	17 (73.9)
<i>s1m2</i>	5 (16.1)	3 (10)	2 (5.8)	7 (18.4)	1 (10)	3 (13)
<i>s2m2</i>	17 (56.7)	18 (60)	16 (47)	10 (26.3)	7 (70)	2 (8.7)
<i>s2m1</i>	2 (6.4)	3 (10)	1 (2.9)	ND	ND	ND
<i>cagA</i> +	29 (93.5)	28 (93.3)	34 (100)	23 (60.5)	2 (20)	21 (91.3)
multiple EPIYA-C motifs	7 (22.5)	17 (56.7)	28 (82.3)	9 (23.7)	ND	5 (21.7)
<i>babA2</i> +	29 (93.5)	29 (96.7)	33 (97.6)	31 (81.6)	10 (100)	22 (95.7)

ND, not detected.

G, Gastritis; GU, Gastric ulcer; PL, Premalignant lesions; DU, Duodenal ulcer.

a. Patients with any stage of gastritis (histopathological analysis) were included in these group.

b. Data include patients with endoscopic diagnosis of GU, but in some of them the subsequent histopathological analysis also evidenced some degree of PL.

Combinations of virulence factors and associations with pathologies

It was observed a significant association between *cagA* and *babA2* genotypes with the presence of *vacA s1* and *m1* alleles ($P < 0.001$) in Cuba. Also in this country, a correlation between the *cagA* and *babA2* status of the strains appeared, but in a lower degree ($P < 0.05$). No correlations between the combinations of virulence factors were observed in Chile, due to the fact that almost all isolates were *cagA*- and *babA2*-positive and a great proportion of them were also *vacA s2m2*. Strains carrying all three virulence factors only had significant association with DU in Cuba ($P < 0.005$)

Discussion

The incidence of severe gastroduodenal diseases varies between geographical areas. These variations have been attributed to environmental conditions, host immunological factors, and differences of specific virulence markers in the *H. pylori* circulating strains^{2,3}. More recently, there is a growing interest to study specific virulence factors in circulating strains of countries and/or populations with different GC rates^{6,7,13,29,30}. The gastric cancer rate and the appearance of gastroduodenal pathologies in Chile and Cuba exhibit large differences; the first one possesses one of the highest GC death rate in the Latin American region and also in the world (20 / 100 000)¹⁸ and the second has one of the lowest GC death rate in Latin American (7.5 / 100 000)¹⁹.

In this study, we found that *cagA* gene was 30 % more prevalent in Chilean than in Cuban isolates. Furthermore, almost half of Chilean *cagA*-positive strains had two or more EPIYA-C motif, while in Cuba only 30.4 % of the isolates had these patterns ($P < 0.05$). Taking these results together and the potential of *cagA*-associated pathogenicity, they may represent important contributors to the differences in GC rates seen between these populations. Both, *cagA* status and the number of EPIYA-C motifs have been linked with cancer prevalence in a number of populations^{7,30}. Additionally, the percentage of *cagA* positivity (64.7 %) in Cuban isolates is closer to the values for Western countries; however the Chilean percentage (94.9 %) resembles the values for East Asia^{3,31,32}. Despite of the differences found, isolates from both countries presented the Western types of *cagA* and consequently the EPIYA-D motif was not found in any isolate studied.

Surprisingly, as was previously reported³⁹ the group of Chilean strains analyzed in this study showed an inverted situation of the expected *vacA* genotypes

for regions in Latin American of high GC rates¹³; the less pathogenic combination *s2m2* was predominant in the Chilean population studied. Even so, a recent study has shown a high proportion of this genotype in a region with populations having elevated GC rates³⁴. However, due to the fact that this phenomenon is new, we are encouraged to perform new studies to repeat and extend our investigation in the near future. In Cuban isolates, the more pathogenic *vacA s1m1* was predominant, with a prevalence slightly higher than that found in Western countries^{35,36}, lower than the reported for Eastern countries³⁶ and in the middle range of Latin American countries^{13,36}.

We looked within the Chilean and Cuban isolates for associations between virulence factors and gastric diseases in the studied groups. In Chile the presence of *cagA* in the strains did not associate with any pathology, due to almost all strains were *cagA*-positive, a situation also observed in several studies from countries and/or populations with the higher GC incidences³. We only found an association between the *cagA* positivity and DU in Cuba, this result is inside to the tendency of positive associations found in previous studies of Cuban dyspeptic patients^{21,27}, and resembles others from Western populations; all, with low rates of premalignant and malignant lesions^{16,38}. In the case of the presence of two or more EPIYA-C motifs and its correlation with pathologies, we found in Chile a significant association with GU and premalignant lesions, with a stronger association in the second case. This last phenomenon has been reported in similar studies of Latin American countries, like Colombia and Brazil^{7,30}; in which, other premalignant and also malignant lesions were associated with the multiplicity of the EPIYA-C motif. It seems to be, as it has been reported already, that the presence of more pathogenic patterns of EPIYA motifs; either, the Western and the Eastern types, leads to the appearance of premalignant and malignant pathologies rather than DU^{29,30,37}. Furthermore, the recent Brazilian study, which included a high number of *H. pylori* strains from patients with GC and DU, showed a strong association between the presence of two or more EPIYA-C motifs and GC but not with DU³⁰. All these evidences together and our results reinforce the theory that there are some bacterial strains that may be more likely to cause DU and others that may promote the development of GC⁴⁰.

The results for *vacA* alleles association with diseases were also assessed; the Chilean group showed a very high number of strains with *vacA s2* genotype (75.6 %), the determining allele for the lowest

VacA cytotoxic activity and therefore virulence³. Consequently, the reported association between the more pathogenic *vacA s1, m1* and *s1m1* genotypes of *H. pylori* strains in countries of high GC rates in Latin American region did not appear¹³. Recently, a high proportion of *vacA s2m2* genotype also appeared in a country with populations of high GC incidence³⁴. Our results suggest that the above statement should be questioned. Cuban isolates showed a typical, and even higher, prevalence of *s1m1* genotypes than the reported for Western strains^{35, 36}. This more virulent genotype only correlated with the presence of DU, a finding observed in a meta-analysis study for the Latin American region¹³. For *babA2*, no significant associations were found between the presence of this genotype and any disease presented in both populations due to the high prevalence of this genotype in the two countries.

Previous reports have shown a clustering of active virulence factors within *H. pylori* strains, for example associations between *cagA*-positive, *vacA s1* and *babA2*-positive genotypes^{16,29}. In agreement with the findings presented in those reports, we found a significant correlation between all these genotypes in Cuban isolates. Additionally, the strains carrying the different combinations of the more pathogenic alleles appeared associated with DU, a result that support the theory that the clustering of virulence factors promotes the cell damage and thus the pathogenicity of *H. pylori*¹⁶. As it was mentioned before, Chilean isolates shown a new clustering of the studied virulence factors, in which the more regular pathogenic association of *cagA*- and *babA2*-positive genotypes was present, but in combination with the less virulent *vacA s2m2* instead of the *vacA s1m1* genotype. This finding is new, but recently a high proportion of strains carrying together the *cagA* gene and *vacA s2m2* alleles were reported in an Alaskan study, a region with populations of high GC incidence⁴¹ as Chile.

Summarizing, in this study we could evidence a high prevalence of virulent strains in both populations

studied in terms of the well-established virulent markers of *H. pylori* and also that isolates are more related to strains from Western countries than to strains from Asian countries. However, there are significant differences between the appearance and combination of these virulent markers. Cuban strains exhibited a relatively high proportion of *cagA*-positive strains, although high percentage of them possessed only one EPIYA-C motif. On the contrary, almost all Chilean strains were *cagA*-positive and almost half of them presented more than two EPIYA-C motifs. The above mentioned results in the *cagA* status from Chile and Cuba appear to be a good partial explanation to the differences in disease prevalence between both countries. Interesting, in the case of *vacA s* and *m* alleles, the group of Chilean strains showed an opposite situation of the expected *vacA* genotypes for regions of high GC rates, a finding that will be study in deep. Also, we will need to include the *dupA* gene status and the *H. pylori* related host immune response in future studies.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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