Abstract

Background: The cytotoxin associated gene A (cagA), and the vacuolating cytotoxin gene A (vacA) of Helicobacter pylori have been associated to phenotypic characteristics of virulence. The objectives of this study were to detect the presence of cagA and to characterize the allelic variants of vacA in 63 strains of H. pylori isolated from colonized individuals with different clinical outcomes.

Methods: 38 strains were isolated from patients with non-ulcerative dyspepsia (NUD) and 25 were isolated from colonized individuals with peptic ulcers. The genotypic characterization was carried out utilizing PCR methodology. The presence of the cagA gene was detected using two set of primers from the middle conservative region of the cagA, and primers for the signal and middle region were used for the genotyping of vacA

Results: The presence of cagA showed similar rates in strains from peptic ulcers (60%) and NUD patients (55%). Also similar was the prevalence of the allelic form s1 of vacA between the strains obtained from ulcers or NUD patients. However, the combination cagA+/vacA s1m1 was found more frequently among the H. pylori strains from peptic ulcer patients (52%) than among strains isolated from NUD patients (26%), this difference was statistically significant (p = 0.035).

Conclusions: The presence of either cagA or the allelic variant s1 vacA alone do not have a predictive value as as a risk markers of severe gastric pathologies in the Chilean population. However, being infected by a H. pylori strain with the genotype cagA+/vacA s1m1 may be associated to an increased risk of acquiring a peptic ulcer disease.

Background

Helicobacter pylori infects about half of the world population, but only a reduced percentage develops peptic ulcers or gastric cancer, both conditions are strongly related with H. pylori infection in Chile [1]. It has been suggested that patients with severe gastroduodenal symptoms are infected with more virulent strains of H. pylori, while those individuals that just develop gastritis are infected with strains of low pathogenic potential. cagA is one of the markers for a pathogenicity island of ca. 40 kilobases, which has been associated to more severe clinical outcomes [2]. vacA codes for another important virulence fac-

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tor that induces vacuolization on eukaryotic cells in vitro, and different allelic variants in two regions of this gene has been described. The N-terminal signal region (s) may occur as the alleles s1 (s1a and 1b) or s2 and the middle region (m) is present as alleles m1 or m2. The variable structure of this gene has been associated with differences in the production of the cytotoxin and the clinical outcome of the \textit{H. pylori} infection [3].

In Chile, two gastroduodenal diseases, peptic ulcers and gastric cancer, are a frequent cause of medical consultation. Moreover, serological studies have indicated that an elevated percentage (over 70%) of asymptomatic adults over 35 years old, are infected by \textit{H. pylori} [4].

The objective of this study was to evaluate the prevalence of the \textit{cagA} gene and the allelic variants of the s and m regions of the \textit{vacA} gene in \textit{H. pylori} strains isolated from Chilean patients.

**Methods**
A total of 63 \textit{H. pylori} strains were analyzed in this study. All strains were isolated from gastric biopsies, 25 from patients with gastroduodenal ulcers, and 38 from colonized individuals with non-ulcerative dyspepsia (NUD). \textit{H. pylori} was identified by means of microscopic observation and urease, catalase and oxidase tests. Isolates were finally confirmed by PCR. The presence of the \textit{cagA} gene was detected by PCR using two set of primers from the middle conservative region of the \textit{cagA}: 5'-GATAACAGGCAAGCTTTTGAGGGA-3' and 5'-CCATGAATTTITGATCCGTCG-3' originating a fragment of 393 bp [5] and 5'-GATAACAGGCAAGCTTTTGAGG-3' and 5'-CTGCAAAAAGATTGTTTGGCAGA-3', which yield a fragment of 349 bp [6]. The genotyping of \textit{vacA} was done by PCR. For the s region, the primers 5'-ATGGAAATACAACAAACACAC-3' and 5'-CTGCTTGAATGCGCCAAAC-3' originated fragments of 259 bp (s1 type) or fragments of 286 bp (s2 type) [3]. For the m region, the primers 5'-CAATCTGTCCAATCAAGCGAG-3' and 5'-GCGTCAAAATAATTCCAAGG-3' originated fragments of 567 bp (m1 type), or fragments of 642 bp (m2 type) [7]. The amplification products were evidenced through 2% agarose gel electrophoresis. Gels were stained with ethidium bromide, and observed under UV light. Comparative statistical analysis was done using the chi-square test.

| Gastroduodenal condition | CagA+ | CagA- | s1 | s2 | m1 | m2 | m1m2 |
|--------------------------|-------|-------|----|----|----|----|------|
| Ulcers*                  | 15 (60%) | 10 (40%) | 16 (62%) | 9 (38%) | 13 (52%) | 11 (44%) | 1 (4%) |
| NUD**                    | 21 (55%) | 17 (45%) | 20 (53%) | 18 (47%) | 14 (37%) | 23 (60%) | 1 (3%) |

*Strains isolated from ulcer patients (N = 25) **Strains isolated from NUD patients (N = 38)

| Gastroduodenal condition | cagA+ | cagA- |
|--------------------------|-------|-------|
| s1m1                     | 13 (52%) | 0 (8%) |
| s1m2                     | 2 (8%) | 0 (4%) |
| s2m2                     | 2 (5%) | 11 (29%) |

*Strains isolated from ulcer patients (N = 25) **Strains isolated from NUD patients (N = 38)
**Results and Discussion**

As shown in Table 1, the presence of cagA was similar in the groups studied, 15/25 (60%) in the strains from ulcer patients and 21/38 (55%) in the strains from NUD patients. The prevalence of the allelic variants of s1 and m1 of vacA was higher in the strains isolated from ulcer patients than in the strains from NUD patients (62% vs 53%, and 52% vs 37% respectively), however these differences were not statistically significant. The combinations s1m1 and s2m2 A were predominant, being s1m1 more prevalent in the strains obtained from ulcer patients (52% in ulcer and 40% in NUD), and s2m2 more prevalent in the strains from NUD cases (36% in ulcer and 47% in NUD), although these differences were not statistically significant. When the cagA and vacA genotypes were combined and analyzed in relation to the clinical outcome (Table 2), the cagA+ strains with the allelic variant s1m1 of vacA were more prevalent in the strains isolated from ulcers patients (52%) than in the strains from NUD patients (26%), this difference was statistically significant (p = 0.035).

Several studies, have demonstrated that the genotype varies among H. pylori strains isolated from different geographic regions [8,9] and conclusions derived from one geographic region may not be true for others. Reports from developed countries have found that the cagA gene and the allelic variant s1 of vacA are more prevalent among strains of H. pylori isolated from patients with peptic ulcers [2,3,10]. In South America, De Gusmao et al. demonstrated an association between the presence of the s1 allele and the presence of duodenal ulcer, and also an association between the presence of the m1 allele of the vacA gene and the presence of peptic ulcers in Brazilian children [11]. On the other hand, reports from other geographical regions like Asian and some Latin American countries have found no association between the presence of cagA or the s1 allelic variant of vacA and the clinical outcome of an H. pylori infection [6,12–15]. Thus, Yamaoka et al. have concluded that no particular combination of iceA, vacA, and cagA is helpful in predicting a patient’s disease status [6]. In our experience, taken individually, the presence of cagA or the allelic variant s1 of vacA do not have a predictive value as risk markers for the development severe gastric pathologies. However, the results presented here are somehow peculiar, since when we considered the prevalence of cagA and the allelic variants of vacA together, the cagA+/s1m1 combination was significantly more prevalent among the strains isolated from peptic ulcer patients than in NUD patients. Although the p value is near the borderline for the statistical significance, these data may reflect a tendency, which are supported by the use of two different sets of primers to evaluate the cagA status. These results then, suggest that in the Chilean population, being infected by a H. pylori strain with the genotype cagA+/vacA s1m1 may be associated to an increased risk of acquiring an ulcer disease. The genetic predisposition of the population, and local environmental factors, may also be important factors in the development of diseases caused by H. pylori, which may explain the differences observed with respect to others Latin American countries. The geographical barriers existing in Chile might in part preserve these differences.

**Conclusions**

The presence of cagA or the allelic variant s1 of vacA alone do not have a predictive value as risk markers for the development of ulcer disease among the Chilean population. However, being infected by a H. pylori strain with the genotype cagA+/vacA s1m1 may increase the risk of acquiring a peptic ulcer.

**Competing interests**

None declared.

**Authors’ contribution**

GZF carried out the genotypification of the H pylori strains by PCR, performed the statistical analysis, participated in the design of the study and drafted the manuscript. MT carried out the isolation of the H pylori strains from patient’s endoscopy samples. GF conceived the study and participated in its design.

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