Disease association with two *Helicobacter pylori* duplicate outer membrane protein genes, *homB* and *homA*

Monica Oleastro¹,², Rita Cordeiro¹, Yoshio Yamaoka³, Dulciene Queiroz⁴, Francis Mégraud*²,⁵, Lurdes Monteiro¹ and Armelle Ménard²,⁵

Address: ¹Departamento de Doenças Infecciosas, Instituto Nacional Saúde Dr Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, Portugal, ²INSERM U853, 33076 Bordeaux, France, ³Department of Medicine, Michael E. DeBakey Veterans Affairs Medical Center and Baylor College of Medicine, 2002 Holcombe Blvd., Houston, Texas 77030, USA, ⁴Laboratório de Pesquisa Bacteriologia, Faculdade de Medicina, UFMG, Av. Alfredo Balena, 190 S/4026 30130-100, Belo Horizonte, Brazil and ⁵Université Victor Segalen Bordeaux 2, Laboratoire de Bactériologie, Bat. 2B RDC Zone Nord, 33076 Bordeaux cedex, France

Email: Monica Oleastro - monica.oleastro@insa.min-saude.pt; Rita Cordeiro - rita.cordeiro@insa.min-saude.pt; Yoshio Yamaoka - yyamaoka@bcm.tmc.edu; Dulciene Queiroz - dqqueiroz@medicina.ufmg.br; Francis Mégraud* - francis.megraud@chu-bordeaux.fr; Lurdes Monteiro - m.lurdes.monteiro@insa.min-saude.pt; Armelle Ménard - armelle.menard@labhel.u-bordeaux2.fr

* Corresponding author

Abstract

**Background:** *homB* encodes a *Helicobacter pylori* outer membrane protein. This gene was previously associated with peptic ulcer disease (PUD) and was shown to induce activation of interleukin-8 secretion *in vitro*, as well as contributing to bacterial adherence. Its 90%-similar gene, *homA*, was previously correlated with gastritis. The present study aimed to evaluate the gastric disease association with *homB* and *homA*, as well as with the *H. pylori* virulence factors *cagA*, *babA* and *vacA*, in 415 *H. pylori* strains isolated from patients from East Asian and Western countries. The correlation among these genotypes was also evaluated.

**Results:** Both *homB* and *homA* genes were heterogeneously distributed worldwide, with a marked difference between East Asian and Western strains. In Western strains (*n* = 234, 124 PUD and 110 non-ulcer dyspepsia (NUD), *homB*, *cagA* and *vacA* s1 were all significantly associated with PUD (*p* = 0.025, *p* = 0.014, *p* = 0.039, respectively), and *homA* was closely correlated with NUD (*p* = 0.072). In East Asian strains (*n* = 138, 73 PUD and 65 NUD), *homB* was found more frequently than *homA*, and none of these genes was associated with the clinical outcome.

Overall, *homB* was associated with the presence of *cagA* (*p* = 0.043) and *vacA* s1 (*p* < 0.001), whereas *homA* was found more frequently in *cagA*-negative (*p* = 0.062) and *vacA* s2 (*p* < 0.001) strains.

Polymorphisms in *homB* and *homA* copy number were observed, with a clear geographical specificity, suggesting an involvement of these genes in host adaptation. A correlation between the *homB* two-copy genotype and PUD was also observed, emphasizing the role of *homB* in the virulence of the strain.

**Conclusion:** The global results suggest that *homB* and *homA* contribute to the determination of clinical outcome.

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Background

*Helicobacter pylori* colonization of the human stomach is associated with chronic gastritis and an increased risk of peptic ulcer disease (PUD), gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma [1-3]. While some *H. pylori*-infected individuals remain asymptomatic, others develop severe gastric disease. Strain-dependent factors may account for differences in clinical outcome, in particular factors that modulate interactions between *H. pylori* and human gastric cells, such as outer membrane proteins (OMP) [4-6]. Recently, the OMP coding gene *homB* was associated with an increased risk of PUD in Portuguese children and young adults (age < 40 years) [7]. Moreover, *in vitro* assays showed that HomB contributes to the proinflammatory characteristics of *H. pylori* and is involved in bacterial adherence, these two phenomena being more pronounced when *homB* is present in two copies in a given strain, compared to one copy only [7]. The *homB* 90%-similar gene, designated *homA*, was found to be associated with non-ulcer dyspepsia (NUD) in that same population [7].

In this study, we investigated gastric disease association with *homB* and *homA*, as well as the *H. pylori* virulence factors *cagA*, *vacA* and *babA*, in a panel of *H. pylori* clinical strains isolated from patients from East Asian and Western countries, presenting different gastric diseases, namely NUD and PUD. The correlation between those bacterial factors was also evaluated.

Results

The presence of *homB* and *homA* in the *H. pylori* clinical strains was determined by PCR. Table 1 summarizes the characteristics of the study population. PCR products, corresponding to either *homA*, *homB* or both genes were obtained for all the 415 strains tested. The presence of both genes in the same genome was detected in 43 strains (10.4%) (36 PUD strains and 7 NUD strains). These strains were excluded from the analysis related to clinical outcome. Thus, a total of 372 strains were included. They comprised 197 strains isolated from PUD patients (66.3% male; 50.3 ± 14.5 years) and 175 strains isolated from NUD patients (53.7% male; 51.1 ± 13.4 years).

**Distribution of homB and homA according to clinical outcome**

The results comparing PUD and NUD strains (n = 372) in different countries are presented in Fig. 1. Overall, *homB* was significantly more prevalent in PUD than in NUD strains (75.9 vs 64.7%, *p* = 0.026, OR = 1.7, 95%CI [1.06–2.74]), a trend also observed in Western strains (61.3 vs 46.3%, *p* = 0.025, OR = 1.84, 95%CI [1.10–3.10]). East Asian strains were predominantly *homB*-positive regardless of the clinical outcome (90.4% in PUD and 83.1% in NUD). Considering the analysis by country (Table 2), *homB* was associated with PUD in strains from France, Sweden, Brazil and Colombia, although with no statistical significance.

Inversely, the *homA* gene was more prevalent in gastritis than in ulcer strains (35.8 vs 25.8%, *p* = 0.046, OR = 1.61, 95%CI [1.06–2.74]), a trend also observed in Western strains (54.6 vs 41.9%), though not significant (Fig. 1). The analysis by country revealed that *homA* was more frequently detected in strains isolated from NUD than from PUD in strains from France, Sweden, Brazil and Colombia, although the difference was not statistically significant (Table 2). Previously, it had been shown that *homB* was strongly associated with PUD strains isolated from young adults (age < 40 years) [7]. In the present study, a total of 90 strains were isolated from this age group. In this group, *homB* was significantly associated with PUD (n = 47, mean age 35.7 ± 5.8 y, 47.8% men) when compared to NUD strains (n = 43, mean age 33.4 ± 5.2 y, 55.9% men) (78.7 vs 48.8%; *p* = 0.006, OR = 3.88, 95% CI [1.41–10.84]). When considering only Western strains (31 PUD and 28 NUD), the same association was found (74.2 vs 35.7%; *p* = 0.007, OR = 5.18, 95% CI [1.49–18.68]), but not when East Asian strains only were considered (data not shown).

It was previously demonstrated that *homB* and *homA* can be present in a single- or two-copy form within a genome [7]. In the present study, the *homB*/*homA* copy number was determined for all 372 strains carrying *homB* or *homA* only. All of the East Asian strains carried the single-copy genotype, and this genotype was also the most frequently found in strains isolated in Portugal (60/100, 60%), France (23/33, 69.7%), Sweden (18/22, 81.8%), Germany (15/19, 78.9%) and Colombia (11/18, 61.1%). Due to the high prevalence of the single-copy genotype, no correlation was found between *homB*/*homA* copy number and clinical outcome in these populations. Regarding strains isolated in the USA, 52.2% (12/23) were found to carry the single-copy genotype, while the remaining carried the two-copy genotype, however the distribution of these genotypes was similar among PUD and NUD strains (data not shown). Finally, concerning strains from Brazil, the two-copy genotype was the most frequently detected (28/37, 75.8%), in both PUD and in NUD strains.

When considering the group of strains isolated from Western young adults (age < 40 years, 31 PUD and 28 NUD), a correlation was observed between copy-number of a specific gene and the clinical outcome. Thus, the *homB* two-copy genotype was the most frequently observed among PUD strains and the rarest genotype among NUD strains (38.7% vs 14.3%, *p* = 0.069), while the inverse situation was observed for the *homA* single-copy genotype.
Table 1: Distribution of *Helicobacter pylori* strains included in the study (n = 415), according to geographical origin and disease status of patients.

| Origin       | Disease | No. of strains | Gender (% male) | Median age ± SD (years) |
|--------------|---------|----------------|-----------------|-------------------------|
| **Western countries** | | | | |
| Portugal     | NUD     | 50             | 44.7            | 51.3 ± 14.6             |
|              | DU      | 36             | 44.4            | 47.6 ± 16.6             |
|              | GU      | 14             | 76.9            | 54.8 ± 14.1             |
|              | Total number | 100 | 47.3           | 51.2 ± 15.1             |
| France       | NUD     | 6              | 100.0           | 38.0 ± 7.8              |
|              | DU      | 28             | 80.0            | 49.3 ± 14.3             |
|              | Total number | 34 | 82.9           | 47.7 ± 14.1             |
| Sweden       | NUD     | 10             | 28.6            | 62.1 ± 6.6              |
|              | DU      | 17             | 80.0            | 69.7 ± 12.9             |
|              | Total number | 27 | 58.8           | 66.6 ± 11.2             |
| Germany      | NUD     | 10             | 40.0            | 57.3 ± 11.0             |
|              | DU      | 10             | 60.0            | 59.8 ± 13.3             |
|              | Total number | 20 | 50.0           | 58.6 ± 11.9             |
| USA          | NUD     | 14             | 57.1            | 41.3 ± 8.8              |
|              | DU      | 15             | 73.3            | 55.6 ± 10.5             |
|              | Total number | 29 | 67.9           | 48.7 ± 12.0             |
| Brazil       | NUD     | 18             | 45.0            | 49.3 ± 13.4             |
|              | DU      | 19             | 35.0            | 50.0 ± 18.8             |
|              | Total number | 37 | 52.4           | 49.7 ± 15.7             |
| Colombia     | NUD     | 9              | 30.0            | 53.0 ± 13.6             |
|              | DU      | 10             | 88.9            | 46.7 ± 11.5             |
|              | Total number | 19 | 57.9           | 50.0 ± 12.7             |
| **East Asian countries** | | | | |
| Japan        | NUD     | 28             | 46.7            | 55.8 ± 16.1             |
|              | DU      | 22             | 59.1            | 40.6 ± 11.5             |
|              | GU      | 21             | 76.2            | 54.4 ± 12.1             |
|              | Total number | 71 | 57.9           | 44.3 ± 12.7             |
| South Korea  | NUD     | 37             | 79.5            | 46.4 ± 10.6             |
|              | DU      | 29             | 70.8            | 45.8 ± 11.9             |
|              | GU      | 1              | *               | -                       |
|              | Total number | 67 | 76.1           | 44.7 ± 9.9              |
| **African country** | | | | |
| Burkina Faso | DU      | 11             | N.A.            | N.A.                    |

NUD, non-ulcer dyspepsia  
DU, duodenal ulcer  
GU, gastric ulcer  
No., number  
N.A., data not available  
* 38 year old male patient

(NUD vs PUD: 35.7% vs 9.7%, p = 0.036, OR = 5.19, 95%CI [1.09–27.87]).

**Distribution of cagA, vacA s and babA according to clinical outcome**

Considering all strains and similarly to *homB*, both *cagA* and *vacA* s1 were independently correlated with PUD (78.9 vs 65.0%, p = 0.014, OR = 2.0, 95%CI [1.16–3.47] for *cagA*; 76.8 vs 67.1%, p = 0.070, OR = 1.6, 95%CI [0.97–2.70] for *vacA*), a tendency also observed in Western strains (72.2 vs 54.6%, p = 0.014, OR = 2.2, 95%CI [1.20–3.86], and 71.0 vs 57.4%, p = 0.039, OR = 1.8, 95%CI [1.05–3.12], respectively). With regard to *babA*, it was only slightly more prevalent in PUD than in gastritis, considering all strains (58.1 vs 54.3%), and the Western strains (47.6 vs 40.7%). East Asian strains were all *vacA* s1, *cagA* and *babA*-positive. Considering the analysis by coun-
try, only the cagA-positive genotype was significantly associated with PUD in strains from Portugal (Table 2).

**Association of homB and homA with cagA, vacA s1 and babA**

The association of homB and homA with the *H. pylori*-virulence genotypes cagA, vacA s1 and babA was also evaluated. Considering only the Western strains, the presence of homB was associated with cagA (p = 0.043) and vacA s1 (p < 0.001), while homA was more frequently found in strains lacking cagA (p = 0.062) and with the vacA s2 genotype (p < 0.001). The East Asian strains were all cagA-positive, babA-positive and vacA s1, among which 94.2% of the Japanese isolates and 78.5% of Korean strains were also homB-positive.

**Discussion**

Using a large panel of *H. pylori* strains (n = 372) isolated from patients from East Asian and Western countries, it was possible to confirm the association of homB with PUD and homA with NUD, previously observed with *H. pylori* strains (n = 84) isolated from Portuguese patients [7]. Considering the distribution according to geographical region, homB was found to be significantly associated with PUD in strains from Western countries. However, when considering each country individually, only a tendency was observed probably due to the small number of strains tested in each case. The most common *H. pylori* strain circulating in the East Asia was extremely virulent, harboring homB, cagA, and babA genes and the vacA s1 genotype, regardless of the clinical outcome. Consequently no association with a specific disease was found, confirming the results from previous studies [8,9]. Indeed, exposure to risk factors must be heterogeneous to find an association, and this is not the case in Asia. In addition, other environmental factors, e.g. diet, and possibly host genetic factors, may contribute to this evolution [10].

The previously reported significant association of homB with PUD in young adults (age < 40 years, 32 patients) [7] was confirmed in the present study with a higher number of patients (n = 90).

Polymorphism in copy number of *H. pylori* OMPs may contribute to increase the fitness of the strain and also its virulence [4,11,12]. Indeed, the homB two-copy genotype...
was associated with an increased rate of in vitro interleukin-8 secretion as well as an increased in vitro adherence [7]. Furthermore, it was the genotype most frequently found in strains from young adults with PUD, while homA single-copy was the most frequent in NUD strains, in agreement with previous data [7]. Globally, these data suggest that in some populations, the severity of *H. pylori*-associated disease in younger subjects may be closely related to the virulence of the strain, irrespective of the contribution of host and/or environmental factors which play a major role in adults. On the other hand, the present study demonstrates that there is a marked geographical specificity regarding homB/homA copy number, particularly evident between East Asian and Western strains, but also amongst Western countries, suggesting that copy number of the homB/homA OMP coding genes also plays a role in adaptation to the human host.

Several *H. pylori* genes encoding OMP display allelic variation, as is the case of babA, babB [13], hopQ [14] and hopZ [15]. In all of these cases, a conserved profile of gene segmentation is observed, with a variable region which defines the existence of at least two highly conserved allelic variants. Regarding homB and homA, no information on allelic variation is available to date. Further sequence analysis of these coding regions using *H. pylori* strains with different geographical background would allow assessing the existence of allelic variation and to evaluate whether different alleles could be associated with a specific clinical outcome and/or reflect a dissimilar geographical origin.

The cagA-positive and vacA s1 genotypes were independently associated with PUD in Western strains, but not babA. Previous publications reported a significant association between the presence of babA and PUD in Western strains [16,17], contrasting with the present result. This discrepancy may be explained by the very heterogeneous Western study group with regard to the geographical origin of the strains, and also because of a possible absence of PCR amplification due to diversity within babA [17,18].

homB was found to co-exist with the most virulent genotypes, while homA was more frequently found in strains lacking these genotypes, in agreement with previous results [7]. Thus, it is likely that the phenotype resulting from the expression of cagA, vacA s1 and homB genesconfers a biological advantage to the strain, with the cumulative action of each factor contributing at the same time to the fitness of the strains in vivo and to a more pronounced

| Table 2: Univariate analysis of the relationship between *Helicobacter pylori* virulence genotypes and clinical outcome according to country, from patients presenting peptic ulcer disease or non-ulcer dyspepsia. |
|-------------------------------------|-------------------------------------------------|---------------------------------|-----------------|-----------------|
| HomB vs vacA s1 vs babA             | P-value†; OR [95%CI]                             |                                |                 |                 |
| HomB                                | HomA                                            | CagA                           | VacA s1         | BabA            |
| Portugal                            | 59.6 vs 56.0                                   | 40.4 vs 44.0                   | 72.3 vs 40.0    | 63.8 vs 44.0    | 48.9 vs 32.0    |
| 50 PUD; 50 NUD                      | N.S.                                           | N.S.                           | N.S.            | N.S.            | N.S.            |
| France                              | 74.1 vs 28.6                                   | 37.0 vs 71.4                   | 85.2 vs 60.0    | 85.2 vs 80.0    | 80.0 vs 50.0    |
| 27 PUD; 6 NUD                       | N.S.                                           | N.S.                           | N.S.            | N.S.            | N.S.            |
| Sweden                              | 41.7 vs 30.0                                   | 58.3 vs 70                     | 83.3 vs 70.0    | 66.7 vs 70.0    | 83.3 vs 40.0    |
| 12 PUD; 10 NUD                      | N.S.                                           | N.S.                           | N.S.            | N.S.            | N.S.            |
| Germany                             | 55.6 vs 60.0                                   | 44.4 vs 40.0                   | 100 vs 80       | 100 vs 80       | 77.8 vs 60.0    |
| 9 PUD; 10 NUD                       | N.S.                                           | N.S.                           | N.S.            | N.S.            | N.S.            |
| USA                                 | 40.0 vs 33.3                                   | 60.0 vs 66.7                   | 60.0 vs 91.7.   | 70.0 vs 91.7.   | 100 vs 91.7.    |
| 10 PUD; 13 NUD                      | N.S.                                           | N.S.                           | N.S.            | N.S.            | N.S.            |
| Brazil                              | 70.0 vs 27.3                                   | 30.0 vs 72.7                   | 60 vs 18.2      | 50.0 vs 27.3    | 100 vs 100      |
| 10 PUD; 12 NUD                      | N.S.                                           | N.S.                           | N.S.            | N.S.            | N.S.            |
| Colombia                            | 77.8 vs 60.0                                   | 33.3 vs 50.0                   | 55.6 vs 80      | 66.7 vs 70.0    | 100 vs 70.0     |
| 9 PUD; 9 NUD                        | N.S.                                           | N.S.                           | N.S.            | N.S.            | N.S.            |
| Japan                               | 95.5 vs 92.9                                   | 47.3 vs 3.6                    | 100 vs 100      | 100 vs 100      | 100 vs 100      |
| 42 PUD; 28 NUD                      | N.S.                                           | N.S.                           | N.A             | N.A             | N.A             |
| South Korea                         | 83.3 vs 73.7                                   | 16.7 vs 26.3                   | 100 vs 100      | 100 vs 100      | 100 vs 100      |
| 28 PUD; 37 NUD                      | N.S.                                           | N.S.                           | N.A             | N.A             | N.A             |

Among the 415 isolates initially included, 43 (36 PUD strains and 7 NUD) harbored both homA and homB genes and were excluded from further analyses. These 49 isolates comprised all the 11 isolates from Burkina Faso.
† p-value was determined by the Fisher’s Exact Test.
OR, odds ratio.
N.S., not significant.
N.A, not applicable.
PUD, peptic ulcer disease
NUD, non-ulcer dyspepsia
pro-inflammatory response. Another hypothesis would be that homB is linked to PUD only because of its association with other virulence factors. However, its role in H. pylori-associated inflammation and in bacterial adherence supports the hypothesis that homB contributes to disease development [7].

Globally, these results suggest that homB and homA seem to be good candidates for the pool of H. pylori factors involved in the determination of clinical outcome.

Methods
Bacterial strains
A total of 415 H. pylori strains isolated from patients from 10 different countries, suffering from NUD (n = 182), PUD (n = 233), of which 197 duodenal ulcers and 36 gastric ulcers, were included in this study (Table 1). H. pylori strains were cultured from gastric biopsies on agar supplemented with 20% horse blood, preserved in trypticase soy broth supplemented with 20% glycerol and maintained at -80°C until used. Genomic DNA was extracted from a 48 h-old culture grown in agar base supplemented with 10% horse blood, using the QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions.

Genotyping of homB, homA, cagA, vacA s and babA by PCR and sequencing
The homB and homA genes were amplified by a single PCR with a set of primers designed on a consensus internal sequence present in both genes [19]. In order to determine the homB and homA copy number, primers targeting the respective loci were used, as previously described [19]. The presence of the vacA s allelic variants, s1 and s2, and cagA and babA genes were determined using published PCR primers [13,16,20,21].

Statistical analysis
Statistical analysis was performed using the statistical software package SPSS (version 14.0; SPSS). The level of significance was set at 5%, with the null hypothesis rejected when p < 0.05.

Abbreviations
(PUD): Peptic ulcer disease; (GU): gastric ulcer; (DU): duodenal ulcer; (NUD): non-ulcer dyspepsia; (OMP): outer membrane protein; (OR): odds ratio; (CI): confidence interval.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
MO carried out the experimental design of the study, statistical analysis and co-drafted the manuscript; RC, YY and DQ carried out bacterial cultures and PCR; FM co-drafted the manuscript; LM supervised the study and AM supervised the study and co-drafted the manuscript. All authors read and approved the final manuscript.

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