High worldwide conservation of a *Helicobacter pylori* outer membrane protein gene, *homD*

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Introduction

Helicobacter pylori **is a gram-negative gastric pathogen possessing a large set of outer membrane proteins (OMPs), which mediate important pathogen-host interactions. The *homD* gene codes for a *H. pylori* OMP and belongs to the *hom* family, together with the recently studied *homB* and *homA* genes. *homB* is implicated in bacterial adherence and in IL-8 activation. No specific function of *homD* is known yet.**

Aim

This work aims to study the genetic diversity and evolution of the *homD* gene, in a large panel of clinical and reference *H. pylori* strains. Moreover, the antigenicity of HomD was also evaluated.

Materials and Methods

Sequences and bacterial strains:

- **26 *homD* sequences from *Hp* complete genome (NCBI).**
- **187 *Hp* clinical strains isolated from patients presenting different gastric disease were used in the analysis:**
  - **68 from Western countries (Portugal: 22, France: 2, Sweden: 12, Germany: 11, Croatia: 1, USA: 7, Colombia: 6, Brazil: 7) presenting non-ulcer dyspepsia and gastritis-G (n=37) , peptic ulcer-PU (n=28) or gastric cancer-GC (n=2); unknown (n=1).**
  - **27 from East Asian countries (Japan: 15, South Korea: 12) presenting non-ulcer dyspepsia and gastritis-G (n=6), peptic ulcer-PU (n=19) or gastric cancer-GC (n=3).**
  - **82 from African countries (Senegal: 72, Burkina Faso: 10) presenting peptic ulcer-PU (n=59) or gastric cancer-GC (n=8); unknown (n=15).**
  - **10 from Oceania (New Caledonia: 10)**

Sequence analysis and phylogeny of homD :

- **The complete sequences of *homD* were obtained by PCR and sequencing;**
- **Bioinformatic analyses. Similarity plots were obtained with *SimPlot* Version 3.5.1 software using the DNA sequence alignments generated by the *BioEdit Sequence Alignment Editor* (Version 7.0.1). Phylogenetic trees were obtained with *MEGA* 4.1 software using the DNA sequence alignments generated by clustalW.**
- **Hydrophilicity, index antigenic and surface probability were analyzed for *homD* amino acid sequences using *Protean* software in the *DNASTAR* package (Version 5.0).**

Preparation of recombinant HomD and immunoproteomics:

- **A recombinant protein HomD fused with glutathion s-transferase (r*Hp*HomD) was constructed by cloning the *homD* ORF of the *H. pylori* reference strain 26695 (HP1453) into the fusion vector pGEX-4T-3 (GE Healthcare). Proteins were separated by SDS-PAGE and probed with pools of sera from *H. pylori*-positive and *H. pylori*-negative patients.**

Results I

- **All but two strains harboured a complete *homD* gene at a conserved locus. *homD* do not seems to be regulated by a slipped-strand mispairing mechanism.**
- **Both phylogenetic reconstruction (Fig. 1) and the similarity plot analysis of *homD* (Fig. 2) point to a high degree of conservation of this OMP among *H. pylori* strains from different geographical regions.**
- **However, a small region (~90 nt) differing between Western strains (group I) and the East Asian, Ameridian, Oceanian and African strains (group II) can be observed in the similarity plot (Fig. 2). Interestingly, this region was also found in some allelic variants of another *hom* family member, the *homC* gene, suggesting the existence of recombination events between these two OMP-encoding genes.**

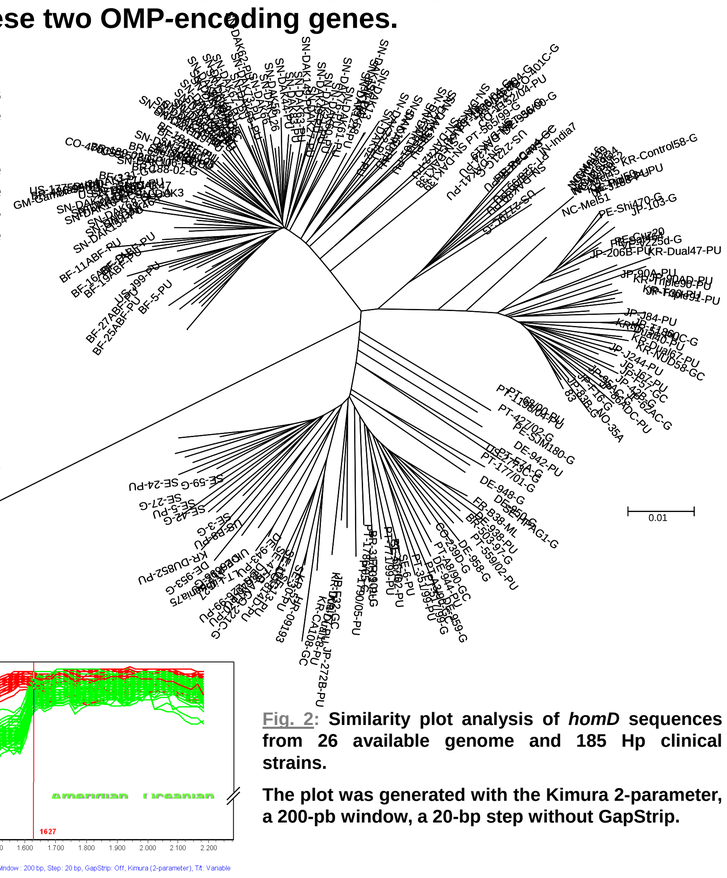

Fig. 1: **Phylogenetic analysis of *homD* sequences obtained from 185 *Hp* clinical and 26 from available genomes.**

The Neighbor-Joining phylogenetic tree of the nucleotide alignments was constructed on the basis of distances estimated using a Kimura 2-parameter model and boostrap 1000 replicates. The branch length index is represented on the tree.

Abbreviations used

BF-Burkina Faso, BR-Brazil, CO-Colombia, DE-Germany, FR-France-FR, GM-Gambia, HR-Croatia, IN-India, IT-Italy, JP-Japan, KR-SouthKorea, LT-Lithuania, US-USA, NC-NewCaledonia, PE-Peru, PT-Portugal, SE-Sweden, SN-Senegal, UK-UnitedKingdom, VE-Venezuela, ZA-SouthAfrica.

G: non-ulcer dyspepsia and gastritis
PU: peptic ulcer
GC: gastric cancer

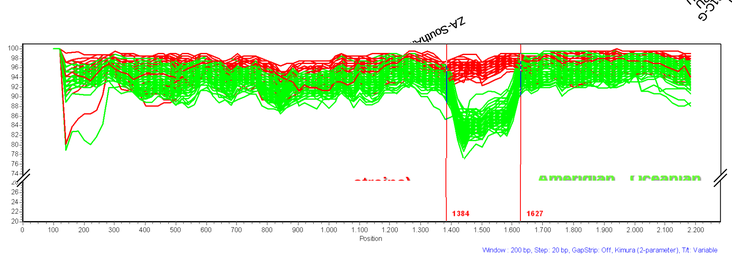

Fig. 2: **Similarity plot analysis of *homD* sequences from 26 available genome and 185 Hp clinical strains.**

The plot was generated with the Kimura 2-parameter, a 200-pb window, a 20-bp step without GapStrip.

Results II

- ***The homD* gene vary from 2223 to 2271 nt and encodes for a 740 to 756 residues protein.**
- ***homD* displayed high similarity at both nucleotide (94.7%) and amino acid (95.9%) levels, corroborating the phylogenetic and the similarity plot analyses. As expected, the frequency of synonymous (Ks) (0.179±0.011) was higher than non-synonymous substitutions (Ka) (0.022±0.002), and Ka/Ks=0.123±0.013. Since Ka/Ks <1, the purifying selection hypothesis was tested and a significant *P value* obtained supports the hypothesis of conservation at the protein level ($P_{Z\text{-}Test}$<0.001).**

Results III

- **Sequence analysis of the HomD predicted protein showed a region located at the N-terminal extremity (residue 58 to 75) with a variable number of Lysine-Proline (KP)-motif repeats (2-9 KP) (Fig. 3A), being the 5 KP-repeat the most prevalent, independently of the geographical origin of the strain. A correlation between the lowest number of KP-motif repeats (≤4 KP) and peptic ulcer disease and the highest number of repeats (≥7 KP) and gastritis was observed, although not statistically significant. No correlation between the number of KP-motif repeats and the virulence of the strain was found.**
- ***In silico* analysis of HomD showed that the region of KP-motif repeats exhibits a strong hydrophilicity (2.71) and antigenicity (6.82) and a high probability of being exposed to the bacterial surface (2.95) (Fig. 3B), displaying considerable higher levels than the adjacent regions (not shown).**

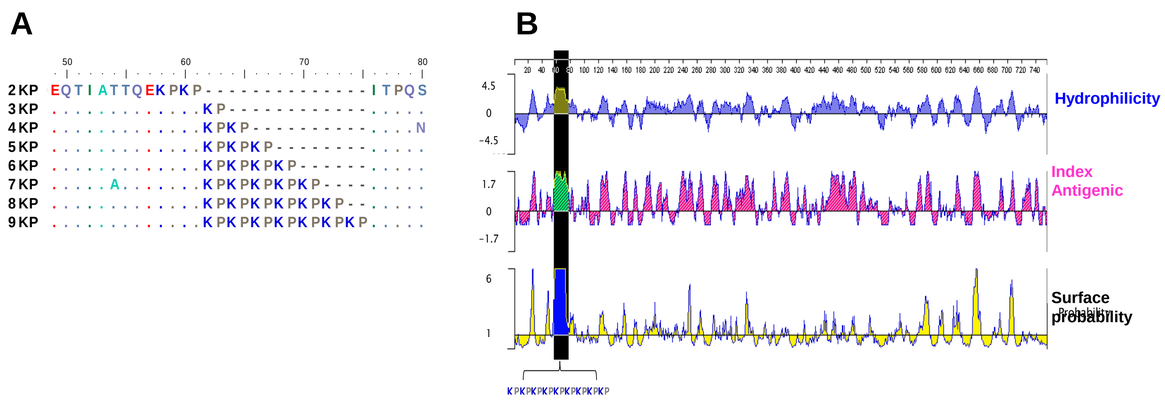

Fig. 3: **(A) Amino acid sequences alignment of the HomD N-terminal showing the Lysine-proline (KP) repeated motifs. (B) Protean analysis of a HomD amino acid sequence.**

Results IV

- **Western Blot analyses demonstrate that HomD protein is antigenic (Fig. 4), corroborating the *in silico* analysis of the amino acid sequences.**

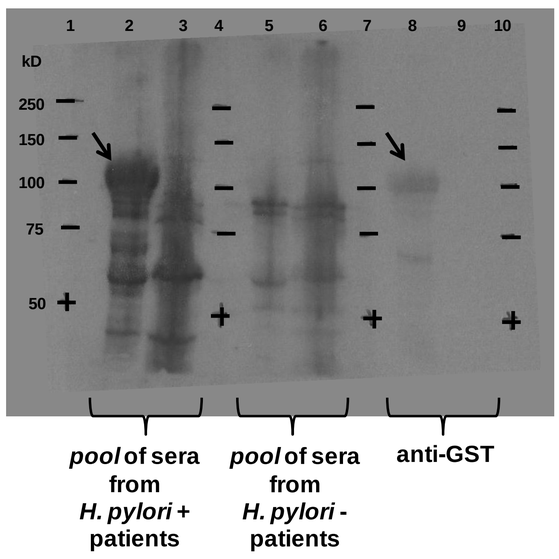

Fig. 4: **Analysis of the seroreactivity of the recombinant HomD protein fused to GST (rHpHomD; Mr=108 KDa).**

Crude extracts of *E. coli* BL21 induced or not by IPTG were analysed by Western Blot. Pool of sera from *H. pylori*–positive and négative patients were used as well as anti– glutathione S-transferase (GST) antibody were used.

Conclusions

Overall, these results show the constant presence of the OMP *homD* in *H. pylori* as well as a high worldwide conservation of *homD*, in contrast to the high polymorphism observed in other *H. pylori* OMPs.

HomD was previouly shown to be present in the membrane fraction of *H. pylori* (Oleastro *et al* 2008 JID 198: 1379-1387) and the present results suggest that HomD gene appears to be an important *H. pylori* antigen, as previously proposed by Meinke *et al* (Vaccine 2009 3251-3259). and because of its high global conservation likely constitutes a new vaccine or therapeutic target.