Abstract no.: P016
Determination of the Prevalence and Genotypes of *H. pylori* in Gastric Biopsy Specimens from Patients with Gastroduodenal Pathologies

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Aim. *Helicobacter pylori* is an important gastric pathogens infects more than 50% of world population. The prognosis of gastric infection could be affected by the production of CagA and VacA toxins and allelic differences in coding genes of these toxins. The aim of this study was to determine the prevalence and genotypes of *H. pylori* in gastric specimens from patients with gastritis, gastric ulcer (G/GU) and duodenal ulcer (DU).
Material and Methods. Two hundred thirty-one patients with gastroduodenal complaints and who are having endoscopic indications were enrolled in this study. The DNA extractions from biopsy specimens were performed with QIAGEN tissue extraction kit. In order to demonstrate *H. pylori* colonization, the spesific sequences of *glmM* gene were amplified with PCR. Then *glmM*-PCR-positive DNA templates were amplified by primers that target spesific sequences in order to detect *cagA* and *vacA* genes.
Results. *H. pylori* was found in 201 (87%) patient's biopsy specimens. The prevalence of *H. pylori* was 82.9% in patients with G/GU and 95.9% in patients with DU. 84.1% of colonized strains were type-I and prevalences of these strains in patients with G/GU and DU were 78.6% and 94.3%, respectively. The most *vacA* allelic combination seen in type-I strains was *s1a/m1b* with 47 (27.8%) strains. Especially, the presence of *s1c* allele in 59 (57.3%) strains from G/GU patients was striking.
Conclusions. Type-I strains of *H. pylori* was found statistically higher in patients with DU than patients with G/GU.

Abstract no.: P017
Detection of Genotype *cagA*, *babA*, and *vacA* Tipification of *H. pylori* in Venezuelan Children with Chronic Gastritis

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Objective. The aim of the study was to determine the presence of different *Helicobacter pylori cagA/vacA/babA* genotypes in the children with chronics gastritis in Venezuela.
Methods. Biopsies were taken from 67 children (1–17 years) at the Department of Gastroenterology, Elias Toro Hospital Caracas, Venezuela. Isolated genomic DNAs from cultured strains were used for genotypes of *H. pylori*. Polymerase chain reactions: two regions of the cagA gene were tested: a region of 349 bp using the primers F1/B1, and a region of 335 bp using the primers B7629/B7628. For vacA gene, we used the VA1-F/VA1-R primers to amplify the 259 bp (s1) or the 286 bp (s2) s region of the conserved portion of the gene, and VAG-R/VAG-F primers to amplify the 567 bp (m1) or the 642 bp (m2) region, and the primers babA2F and babA2R for babA2 gene.
Results. Of 67 patients, 25% were positive to *H. pylori* culture and 42% negative to culture. The genotypes were determined in 10 strains; the presence of the gene cagA was detected on 100% of the patients. For *vacA* gene, the predominant combination (90%) was *m1s1* in contraposition to 10% for *vacAs2m2* ($p > .0001$), and babA2 was positive in the 30% of de strains. The genotype for the strains studied was 60% *cagA+*, *vacAs1/m1*, *babA–*, 30% *cagA+*, *vacAs1+ babA2+* and 10% *cagA+*, *vacAs2/m2*, *babA–* ($p < .001$). Venezuelan strains of *H. pylori* showed a high genetic variability and virulent genes associated distinctiveness in symptomatic population. Grant for LOCTI-IBA-02. FONACIT G-2005000371.

P03 Virulence Factors and Pathogenesis

Abstract no.: P018
H. pylori rocF Is Essential for Gastric Colonization in C57BL/6 and iNos$^{-/-}$ C57BL/6 Mice

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Helicobacter pylori rocF encodes arginase that can hydrolyze L-Arg to L-ornithine and urea. This process could reduce the availability of gastric L-arginine (L-Arg) as a substrate of iNOS for generating NO, a reactive oxygen species with bactericidal effects, which may contribute to persistent *H. pylori* colonization of the stomach. In this study, two *rocF*-deficient isogenic mutants (*rocF*Δcat1 and *rocF*Δcat15) of *H. pylori* strain SS1 were generated and used to infect female 9-week-old C57BL/6 mice in parallel with their parental strain. At 4 or 16 week postinoculation (WPI), all mice infected with *H. pylori* SS1 (10/10 at each time-point) were colonized by the bacterium measured by qPCR. By contrast, the mice (20/20 at each time-point) infected with both *rocF*Δ mutants were negative for *H. pylori*. At 16 WPI, histopathologic activity index (including gastritis, atrophy, hyperplasia, and dysplasia) for the stomachs of *H. pylori*-infected mice was significantly higher ($p < .0001$) than mice infected with either of the *rocF*Δ mutants or the sham controls. To investigate whether clearance of these *rocF*Δ

mutants by the host is due to increased iNos production, *rocF*Δcat15 was used to infect *iNos*$^{-/-}$ C57BL/6 mice that cleared the mutant by 12 WPI, whereas SS1 colonized the stomachs of the infected *iNos*$^{-/-}$ mice. Thus, our data collectively demonstrate that this enzyme is required for gastric colonization of these mouse strains, presumably via acidic survival of *H. pylori* in murine stomachs.

Abstract no.: P019
Helicobacter hepaticus Urease Is Not Required for Intestinal Colonization but Promotes Hepatic Inflammation in Male A/JCr Mice

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Urease activity contributes to bacterial survival in the acidic environment of the stomach and is essential for persistent infection by known gastric Helicobacters such as the human pathogen *Helicobacter pylori*. Several enterohepatic *Helicobacter* species (EHS) that primarily infect the less acidic intestine also have very active urease enzymes. The importance of urease and its contribution to pathogenesis for these EHS are poorly understood. In this study, we generated a urease-deficient, isogenic mutant (*HhureNT9*) of *Helicobacter hepaticus* 3B1 (Hh 3B1), an EHS that possesses a urease gene cluster similar to that of *H. pylori*. Lack of urease activity did not affect the level of cecal colonization by *HhureNT9* compared to Hh 3B1 in male A/JCr mice ($p = .48$) at 4 months postinoculation (MPI). In contrast, there was no *HhureNT9* detected in the livers of any infected mice, whereas all livers from the *Hh* 3B1-infected mice were PCR-positive for Hh 3B1. The mice infected with *HhureNT9* developed significantly less severe hepatitis ($p = .017$) and also produced significantly lower hepatic mRNA levels of proinflammatory cytokines IFN-γ ($p < .001$) and TNF-α ($p < .0001$) compared to the Hh 3B1-infected mice. The Hh 3B1-infected mice developed significantly higher total IgG, Th1-associated IgG2a and Th2-associated IgG1 responses to infection. These results indicate that *H. hepaticus* urease activity plays a crucial role in hepatic disease but is not required for cecal colonization by *H. hepaticus*.

Abstract no.: P020
cDNA Microarray Detection of pH-dependent Gene Expression in *H. pylori* Strains from Different Pathologies

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Because the stomach is a constantly changing environment, successful colonization by *Helicobacter pylori* of this niche requires regulation of bacterial gene expression to cope with environmental fluctuations, particularly pH. To extend our knowledge of the process of regulation and adaptation of *H. pylori* to pH, which is known to oscillate within the gastric mucosa, we undertook an investigation to identify genes whose expression are dependant on changes in pH using cDNA microarrays. *H. pylori* strains 26,695 (gastritis-associated), J99 (ulcer-associated), and AG-1 (atrophic gastritis-associated) were grown at 37 °C under microaerobic conditions in Brucella broth containing 10% horse blood serum. Bacteria from such liquid cultures ($OD_{550} = 0.7$) were harvested, re-suspended in medium whose pH was adjusted to pH 5.0 and pH 7.0, and incubation continued for a further 1-hour period. RNA extraction and cDNA synthesis were performed using standardized protocols and Invitrogen commercial kits. cDNA probes were hybridized with an *H. pylori* microarray (Bacterial Microarray Group, University of London). Genes were identified whose expression was acid pH-dependent in all the strains; 68 genes were up-regulated, and as would be expected, these included urease-related genes (*ureA*, *ureB*, *ureI*, and *UreF*), but also genes involved in flagellar synthesis (*flgB*, *fliM*, and *flgE*). Additionally, up-regulation of genes encoding transport and binding proteins (*glnQ*, *glnH*, and *exbD* and the glutamine-rich metal binding protein gene) were observed. Furthermore, 37 genes were down-regulated from different functional groups. In summary, pH-dependent gene expression was similar in all three strains from differing pathologies.

Abstract no.: P021
Bioinformatics Analysis of Differentially Regulated Genes of *H. pylori* in Response to Acid Stress

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As a colonizer of the human stomach, *Helicobacter pylori* is subject to environmental stress, and to evaluate bacterial RNA expression in response to acid stress we compared available data from two experimental works using advanced bioinformatic analytical tools. Raw data sets from both experimental investigations were obtained from the Gene Expression Omnibus microarray database. Gene lists were annotated using TIGR annotation and the publically available analysis tool, EASE. For data normalization, signal values were imported into GeneSpring 5.0 software and data intensities converted to $\log_2$ for statistical analysis. After this normalization, genes showing significant up- or down-regulation (twofold) were identified by considering the gene expression changes at pH 5.0 (0 minute versus 60 minutes) for wild-type strains (data set 1) and for a wild-type and knockout mutant in AraS (histidine kinase, required for colonization) (data set 2). Based on gene expression profiling of the first data set, 96 genes showed over-expression and 80 genes under-expression. In data set 2, a total of 101 genes showed over-expression and 378 genes under-expression (considering multiple *t*-tests, false discovery rate, and $p < .05$). Comparing the gene expression pattern of both data sets, 29 common genes were identified as differentially expressed, of which 16 genes were

up- and 13 genes were down-regulated. Importantly, for the first time, analysis allowed identification of genes encoding metal-regulation and uptake systems, and those encoding certain glycosyltransferases, that are putatively involved in the acid response of *H. pylori*.

Abstract no.: P022
CagA Protein Diversity with Respect to EPIYA Phosphorylation Motifs in *H. pylori* Infected Children – Relation to Histopathology

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EPIYA tyrosine phosphorylation motifs (TPMs) *A*:EPIYAKVNK, *B*:EPIYAQVAKK and *C*:EPIYATIDDLG in CagA protein of *Helicobacter pylori*, and in particular the presence of increasing numbers of repeating C-motifs has been linked to heavier cagA-dependent pathogenicity in adults. Our aim was to assess whether EPIYA diversity was related to the severity of histopathology in a pediatric population. Clinical isolates from children (n = 94, mean age 10.7 years old ± 0.3) were analyzed by amplification and sequencing of the 3′ variable region of cagA gene. Empty-site-cagA PCR was used for the detection of cagA-negative strains. Clonal relatedness was analyzed by RAPD PCR and MLST analysis. *H. pylori* colonization and associated gastritis was evaluated by the modified Sydney system.

Eighty-one cases (86.2%) were found to be single-strain infections, with 39.5% being attributed to *cagA*-negative strains. CagA-positive strains harbored one or two EPIYA-C repeats (46.9% and 13.6%, respectively,) whereas no strains with more than two repeats were detected. In 13 cases, mixed isolate infections were observed with simultaneous presence of *cagA*-negative and -positive clones with one and two EPIYA repeats, derived from microevolution of the initial infecting *H. pylori* strains. Histological analysis revealed marked chronic inflammatory infiltration in 39 cases (42.9%) and marked chronic active gastritis (n = 7, 7.7%) in the antrum. No significant positive association was observed between EPIYA diversity, levels of *H. pylori* colonization, or the grade and severity of associated gastritis in the antrum. Thus, EPIYA diversity in CagA protein is not related to histopathological parameters in infected children. However, no strains with more than two EPIYA-C repeats were observed.

Abstract no.: P023
Contribution of the *H. pylori* Outer Membrane Protein Encoding Gene *hom*B to Bacterial Adherence

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Background. *hom*B encodes a *Helicobacter pylori* outer membrane protein (OMP). This gene was previously associated with peptic ulcer disease and was shown to induce activation of Interleukin-8 secretion in vitro.
Aims. The present study aimed to elucidate the mechanism underlying the involvement of *hom*B in *H. pylori* inflammation.
Methods. Seven *H. pylori* clinical strains and the corresponding *homB* knock-out mutant strains, 6 single mutants, and 1 double mutant were tested using in vitro adherence assays. The bacterial suspensions were labelled with a PKH2 Green Fluorescent Linker Kit (Sigma, Saint-Quentin Fallavier, France) and, after 18 hours of coculture of *H. pylori* strains with human gastric epithelial AGS cells (ATCC CRL-173), fluorescence was measured by flow cytometry (FACSCalibur Flow Cytometer).
Results. All of the *H. pylori* wild-type strains tested adhered to human gastric epithelial cells, whereas the corresponding single copy *hom*B knock-out mutant strains showed significantly reduced binding. This decrease was more pronounced when the two copies of *hom*B were disrupted, compared to the corresponding single mutant strain.
Conclusions. These results strongly suggest that *hom*B is involved in adherence to human gastric epithelial cells in vitro and that this function is correlated with the number of copies of *hom*B present in a strain. However, the fact that the disruption of *hom*B did not completely abolish adherence, suggests that HomB is not the major OMP involved in this mechanism.

Abstract no.: P024
Gastric Epithelial Cell Apoptosis in Patients with Chronic *H. pylori*-associated Antrum Gastritis among Native and Alien Inhabitants of Eastern Siberia

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Aim. To study epithelial cell apoptosis parameters, atrophy rate and *H. pylori* dissemination parameters in gastric antrum mucosa in various ethnic groups of the Eastern Siberia population.
Methods. We examined 23 Evenks, 23 Khakases, and 22 Europoids with histologically confirmed gastritis aged 18 to 50 years. All subjects underwent upper digestive tract endoscopy,

and antrum mucosa biopsy specimens were taken. Morphological research included microscopic examination after staining by hematoxylin and eosine with the description of results using a visual-analog scale and definition of *H. pylori* dissemination parameters after Gimsa staining. Epithelial cell apoptosis in gastric antrum mucosa was determined by the TUNEL method (Mebstain Apoptosis kit direct, Immunotech, France). Apoptotic index (AI) was determined by counting the percentage of TUNEL-positive epithelial cells at ×400 magnification.

Results. In gastric antrum mucosa AI was 5.02% in Europoids (group 1); 4.7% in Khakases (group 2); 2.67% in Evenks (group 3); $p_{1-3} < .05$; $p_{2-3} < .05$. Rate of atrophy in gastric antrum was 25.2% in group 1; 14.3% in group 2; 10% in group 3 ($p_{1-2} = .05$, $p_{1-3} = .02$). *H. pylori* density dissemination in gastric antrum mucosa in Europoids was higher than in Mongoloids: 206.4 in group 1; 125.1 in group 2; 126.6 in group 3 ($p_{1-2} < .001$; $p_{1-3} < .001$).

Conclusions. High apoptotic index in Europoids was associated with high rate of antral atrophic gastritis and high *H. pylori* density dissemination in comparison with in different ethnic groups of Mongoloids.

Abstract no.: P025
cagE not *dupA* Plays an Important Role in IL-8 Secretion In Vitro

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The induction of IL-8 secretion by AGS cells after coculture with *Helicobacter pylori* in vitro has been associated with the presence of *cagE* and *dupA*.

Methods. *H. pylori* was cultured from biopsies from Malaysian and Singaporean subjects diagnosed with functional dyspepsia (FD) (Chinese n = 51, Indian n = 46, Malay n = 14), duodenal ulcer (DU) (Chinese n = 13), or gastric cancer (GC) (Chinese n = 22). Polymerase chain reaction was used to determine the prevalence of *cagE* and *dupA* in isolates. Twenty-one isolates (7 *cagE+dupA+*, 12 *cagE+dupA–*, 1 *cagE–dupA+* and 1 *cagE–dupA–*) were cocultured with AGS cells for 6 hours before IL-8 secretion was measured by ELISA.

Results. Of Chinese FD, DU and GC isolates, 94%, 100%, and 95% and 93% of Indian and 100% Malay FD isolates possessed *cagE*. *dupA* was detected in 29%, 63%, and 55% of Chinese FD, DU, and GC isolates and 7% and 38% of Indian and Malay FD isolates. IL-8 secretion by AGS cells was highest with *cagE+dupA+* (1330 ± 394 pg/mL) and *cagE+dupA* (1378 ± 165 pg/mL) isolates. The *cagE–dupA+* and *cagE–dupA–* isolates secreted 394 pg/mL and 165 pg/mL IL-8, respectively.

Conclusion. *cagE* prevalence did not vary significantly between ethnic groups or disease status. *dupA* prevalence was significantly higher in isolates from ethnic Chinese DU and GC subjects and lower in isolates from ethnic Indian FD subjects, than other groups ($p < .05$). IL-8 induction in vitro was associated with *cagE* but not *dupA* in the isolates investigated, a finding that would suggest that the contribution of *dupA* to IL-8 induction may be less than previously suggested.

Abstract no.: P026
A Homology Model of *H. pylori* OMPLA

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Introduction. Outer membrane phospholipase A (OMPLA) is a membrane-bound enzyme that degrades bacterial phospholipids to lysophospholipids. *H. pylori* OMPLA activity correlates with ulcer disease in patients. The aim of this study is to analyze the model a *H. pylori* OMPLA structure in order to yield a better understanding of activity and regulation.

Methods. A homology model was built with the WHAT IF software using the PDB 1FW3.pdb (from *E. coli*) as a template (percentage sequence identity: 35%). The active site residues were protonated and the N-terminus was acetylated. The modelled protein was inserted in a large solvated POPE lipid membrane and ions were added to neutralize the system. After energy minimization of the complete system with a classical force field, the modelled protein was kept fixed and the surrounding membrane and ions equilibrated using molecular dynamics (MD) simulations. A 100-ns MD of the fully equilibrated system was then performed.

Results and Discussion. The most conserved regions predicted to be necessary for activity were maintained in the model. Two inserts of *the H. pylori* sequence have been omitted from the model. One is predicted to be a signal sequence at the N-terminal end. The other insert is *Helicobacter*-specific and is located at one of the exterior loops. MD simulation of the model shows a stable structure when the flexible loops are not taken into account. These differences partly seem related directly to structural characteristics, but partly also to the dynamic behavior.

Abstract no.: P027
Association of *H. pylori* Genotypes and Cytokine Genes (IL-1) Polymorphisms with Morphometric Characteristics of Ulcers

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Although *Helicobacter pylori* infection affects gastric and duodenal ulcer healing, cofactors involved in the establishment of final size of gastric or duodenal ulcer and in ulcer healing still remain unclear. IL-1 plays a crucial role in ulcer healing by up-regulation of several healing-related factors expression.

The purpose of our study was to evaluate gene polymorphism of proinflammatory (IL-1β) cytokine in patients with *H. pylori*-associated gastric and duodenal ulcer disease with healed ulcers (43 persons) and with disease recrudescence (78 persons). The disease recrudescence group was divided according to ulcer sizes. IL-1B-511/+3954 and IL-1RN were assessed by polymerase chain reaction (PCR) and restriction fragment-length polymorphism (RFLP) analysis. The presence of bacterial virulence factors was investigated by cagA, vacAs1/s2/m1/m2 PCR.

Strong association between vacAs1-positivity and disease recrudescence was found ($p < .001$). No association was found between cagA-status of *H. pylori* and ulcer sizes. The presence of IL-1RN2/2 genotype had strong association with the development of small ulcers (less than 5 mm) ($p < .017$), whereas IL-1B-511/+3954 polymorphism was not statistically different in groups with different ulcer sizes. These data show that IL-1RN polymorphism might be linked to ulcer occurrence at initial stage.

Abstract no.: P028
Characterization of CagA Variable Region of *H. pylori* Isolates from Iranian Dyspeptic Patients

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Introduction. CagA is associated with an increased risk of peptic ulceration, atrophic gastritis, and noncardia gastric cancer. The biological activity of CagA protein is related to the variable region of CagA containing variable numbers of EPIYA motifs. CagA is subdivided to the East Asian and Western subtypes based on its binding to SHP-2. The aim of this study is to characterize the CagA variants of Iranian *H. pylori* strains and their association with the infection associated diseases.

Methods. CagA variable region of 172 isolates recovered from 112 NUD, 33 PUD and 27 GC cases were amplified using cag2 and cag4 primers. All the obtained partial nucleotide *cagA* sequences in this study were deposited in the GenBank.

Results. Eighty one percent of the examined isolates possessed *cagA* gene. Six CagA variants differing in length were identified. Two to six EPIYA motifs were detected among the sequenced strains. The most prevalent (45.9%) amplicon was 550 bp designated as A-B-C type. All of the sequenced CagA variable regions were identified as the "Western" type. There was no statistical association between any of the CagA subtypes and gastrointestinal disorders.

Conclusion. The primary study showed that Iranian stains have a lower binding affinity to SHP-2 than other East Asian strains. It is suggested that circulation of A-B-C type as the most prevalent type in Iranian dyspeptic patients and lower binding affinity of our strains to SHP-2 may have resulted in lower frequency of gastric cancer in our population compared to East Asian countries.

Abstract no.: P029
CagA-positive Strains of *H. pylori* Impair Trophoblast Cells Invasiveness: Do They Play a Role in Pre-eclampsia and Poliabortivity?

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Background. The role of bacterial and viral infection in trophoblast diseases such as pre-eclampsia and poliabortivity has been extensively studied in the past few years. Although trophoblast cells show an endothelial phenotypic profile, a study from our group showed that antibodies anti-CagA cross-react with endothelial cells, possibly playing a role in some vascular diseases; we have hypothesized that CagA-positive strains of *Helicobacter pylori* may also recognize antigens of trophoblast cells, thus impairing their invasion capability.

Methods. Placenta samples were obtained from healthy women immediately after uncomplicated vaginal delivery at 36 weeks of gestation. Five isolated trophoblast cell lines were cultured for 72 hours in a medium containing increasing concentration of polyclonal anti-CagA antibodies (from 6 to 200 μg/mL). Binding of anti-CagA antibodies to trophoblast cells was verified through flow cytofluorimetry, while the invasive potential of these cells was assessed using a membrane invasion culture system.

Results. Anti-CagA antibodies recognized antigens of trophoblast cells of all samples, showing a dose-dependent binding up to the highest concentration of 200 μg/mL. Interestingly, incubation of trophoblast cells with increasing doses of anti-CagA antibodies significantly reduced their invasiveness.

Conclusions. This preliminary study reports, for the first time, that anti-CagA antibodies are able to recognize antigens expressed on the surface of trophoblast cells, and to reduce their invasiveness ability. Interestingly, the inhibitory effect of anti-CagA antibodies on trophoblast cells is directly related to the concentration of the same antibodies. Further studies are now needed in order to identify the cross-reactive antigens responsible for this phenomenon.

Abstract no.: P030
Comparative Proteomic Analysis of *H. pylori* Clinical Isolates

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Helicobacter pylori is the etiological agent of gastric disorders. This pathogen presents a high level of genetic variability among strains which, in addition to host's factors, determine the course and the outcome of infection. A comparative proteomic analysis of *H. pylori* strains clinically isolated from colonized patients but still with normal mucosa and those with peptic ulcers, gastritis, and

gastric cancer were chosen to identify strain's specific proteins. These are strain's biomarkers, whereas conserved and highly immunogenic proteins among these strains will function as targets for therapeutics and vaccine purposes. Bacterial total protein extracts were analyzed by two-dimensional gel electrophoresis (2DE) being isoelectric focused onto nonlinear pH 3–11 gradient-strips (GE-Healthcare) and then separated on a 7–16% (w/v) SDS-PAGE. Proteins were visualized by coomassie-staining. 2DE-gels digitalized images are being analysed using ImageMaster™ 2D-Platinum software (Geneva-Bioinformatics, SA). Although the proteome from such strains is quite similar, there are indeed some proteins whose expression profile is correlated to the particular *H. pylori*-associated gastric disorder. These proteins identification by mass spectrometry will contribute to elucidate the mechanisms underlining virulence, becoming valuable for prognosis, diagnosis and therapeutics of *H. pylori* infections. Immunoreactive conserved protein targets are being determined by 2DE-gels immunoblotting with a pool of antibodies anti-*H. pylori* (commercially available). These are good candidates to include on a DNA-based broad spectrum vaccine. We thank Professor Lurdes Monteiro (INSA, Portugal), Professor Filipa Vale (FEUCP, Portugal) and Professor Jorge Vitor (FFUL) for the strains. Work supported by PTDC/BIO/69242/2006 research grant. IV is recipient of SFRH/BD/38634/2007 doctoral fellowship.

Abstract no.: P031
Induction of IL-8 Production by *H. pylori* Strains with Different *cagA* Genotype and *oipA* Functional Status

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IL-8 is a potent neutrophil chemotactic and activating proinfiammatory cytokine, thought to be related to the mucosal infiltration with neutrophils and mononuclear cells characteristic of the *H. pylori*-related gastritis, and suggested to have a role in *H. pylori*-associated gastroduodenal diseases. Its induction by *H. pylori* strains is increased if a functional *cag* pathogenicity island (PAI) is present in the *H. pylori* strains or if their outer inflammatory protein (*oipA*) gene is in the functional on-status. The *cagA* positivity, expression of the presence of the *cag* PAI, and the *oipA* on-status are characters usually considered to be well correlated with each other.

During a recent study on the *H. pylori* virulence genotypes circulating in Western Sicily, Italy, we isolated some strains in which the presence of *cagA* was associated with the presence of *babA2 and vacA*s1 and *vacA*m1 alleles, but not with the *oipA* on-status, evaluated by DNA sequencing, on the basis of the number of the CT dinucleotide repeats in the 5′ region of the gene.

Mainly to obtain evidence of the phenotypic functional status of their *oip*A genes, the Sicilian strains were cocultured with AGS gastric cancer cells and the IL-8 production was assayed by ELISA.

Results are discussed with respect to the hypothesis that mutations in the promoter region of the *oipA* gene can prevent the switch effect of the CT repeats on the expression of a functional open reading frame.

Abstract no.: P032
vacA and cagA Genotypes of *H. pylori* Isolates from First-Degree Relatives of Patient with Gastric Cancer

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Introduction. *Helicobacter pylori* predisposes infected patients to gastric cancer by inducing precancerous alterations. First-degree relatives of cancer patients (FDRCPs) have been found to be at increased risk of developing gastric cancer. Reports suggest that clinical outcome of *H. pylori* infection could be related to certain genetic markers, e.g. *cagA* and *vacA* genes. This study was designed to determine the presence of *cagA* gene and variations in *vacA* alleles in *H. pylori* isolates from FDRCPs.
Methods. Fifty *H. pylori* strains were isolated from gastric biopsies of 50 FDRCPs (40: nonulcers and 10: ulcers). Bacterial strains were identified by biochemical tests and amplification of *H. pylori*-16S rDNA. Primers were designed for amplification of the *cagA* and *vacA* signal sequence and middle region alleles.
Results. Heterogeneity of *cagA* and *vacA* alleles among 50 *H. pylori* isolates from FDRCPs.

	S1/m1	S1/m2	S2/m1	S2/m2
cagA (+) (38)	23/50 (46%)	9/50 (18%)	5/50 (10%)	1/50 (2%)
cagA (–) (12)	5/50 (10%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Total	28/50 (56%)	12/50 (24%)	6/50 (12%)	4/50 (8%)

Discussion. *cagA*$^+$ *H. pylori* strains with the *vacA* s1/m1 genotype were predominant in FDRCPs; s1/m2 was the second, s1/m2 and s2/m2 genotypes had low prevalence. We could not find any association between genotypes of *H. pylori* strains and clinical outcomes in patients. Our results were similar to those found in East Asia (s1/m1, predominant), whereas *vacA* s1/m2 was predominant in Turkey, similar to northern and eastern Europe. Further studies with more isolates might help to predict the clinical outcome of *H. pylori* infection, with certain genotypes, and the consequences in individuals at high risk of gastric cancer.

Abstract no.: P033
Clinical Implications and Prevalence of *cagA*, *cagE*, and *cagT* genes in the Pathogenicity Island of Irish *H. pylori* Strains

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Introduction. *Helicobacter pylori* is the major etiological agent in the development of chronic gastritis, duodenal ulcer, and gastric carcinoma in humans. Pathogenicity islands (PAIs) encode various

virulence factors. The aim is to analyze the genes of *cag* PAI (*cagA, cagE*, and *cagT*) of *H. pylori* for their presence and correlating them with the disease status in patients with intestinal metaplasia (IM) and chronic gastritis (CG).

Materials and Methods. Gastric biopsies of antrum and corpus were collected from *H. pylori*-positive patients, and classified to IM (n = 39) and CG (n = 39) based on histological finding. DNA was extracted and *cagA, cagE*, and *cagT*, amplification were identified by polymerase chain reaction (PCR).

Results. The *cagA, cagE, and cagT* were identified in 83%, 79%, and 69%, respectively, of *H. pylori* strains. Three of 39 (8%) of IM group (*H. pylori* from A and C) similar genotyping for the three genes, but 2 of 39 (5%) of IM patients (*H. pylori* from A) different with cagA, (*cagA* positive *cagE, cagT* negative), (*cagA* negative *cagE, cagT* positive). However, 8 of 39 (21%) of CG group (*H. pylori* from A&C), and 12 of 39 (31%) of CG with different genotyping between the three genes.

Table: Distribution of *cagA*, *cagE*, and *cagT*

Disease	CG (n = 39)	IM (n = 39)	*p*-value
cagA	31(79%)	34 (87%)	
cagE	27 (69%)	35 (90%)	
cagT	20 (51%)	34 (87%)	< .001

Conclusions. Genotyping by PCR, *cagT* might be an important locus of the cagPAI, thus greatly influencing the disease condition of the subject. Patients of chronic gastritis can be colonized with more than one *H. pylori* strains (31%).

Abstract no.: P034
Genotyping *cagA* and *vacA* of *H. pylori* Isolates from Patients with Gastrointestinal Diseases

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Background. *Helicobacter pylori* has been acecepted as the causative agent of gastrointestinal diseases such as gastritis, peptic ulcer, gastric cancer and mucosa-associated lymphoid tissue lymphoma.

Aims. The aim of this study is to evaluate the relationship between major virulence factors (*vacA* and *cagA*) of *H. pylori* and the gastrointestinal disorders.

Materials and Methods. Thirty-four stock strains were included in the study. The DNA templates of the isolates were extracted by the cetyltrimethyl-ammonium bromide (CTAB) method. The presence of *vacA* alleles and *cagA* was determined by polymerase chain reaction (PCR) amplification. Primers VA3-F VA-3R, VA4-F VA4-R, SS1-F VA1-R, SS2-F VA1-R were used for the amplification of 290 bp, 352 bp, 190 bp, 199 bp regions of *vacA* alleles m1, m2, s1a and s2, respectively. For the detection of *cagA*, a 348-bp internal fragment of the gene was amplified by using the F1 ve B1 primers. *H. pylori* NCTC 11,637 was used as a positive control; sterile distilled water was the negative control. PCR products were resolved on a 1.3% agarose gel, stained with ethidium bromide and visuilized on UV light.

Results. The presence of the *vacA* and *cagA* genes and gastrointestinal diseases of the patiens is shown in the table.

Gastrointestinal diseases	*cagA* (n)	*vacA* s1a m1 (n)	*vacA* s2 m2 (n)	*vacA* s1 m2 (n)
Gastritis (n : 5)	5	5	0	0
Duodenal ulcer (n : 5)	3	3	1	1
Antral nodularity (n : 18)	12	11	5	2
Hiatal hernia, esophagitis (n : 1)	1	1	0	0
Normal (n : 5)	4	3	1	1

Conclusion. The *cagA* and s1a m1 genotype of *vacA* was detected in 76% and 68% of the patients, respectively, and the presence of these genes is associated with various gastrointestinal diseases.