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Distribution of virulence marker of Helicobacter pylori patients with functional dyspepsia in Pakistan

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Abstract no.: 02.15
Study of the Transcription Machinery and Gene Regulation in *Helicobacter pylori*

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We are interested in the transcription machinery (RNA polymerase and transcription factors) and the mechanism of transcription in *Helicobacter pylori*. Much progress has been made in the past years in elucidating the in vivo regulation of gene expression in *H. pylori*. However, there is no in vitro transcription system available to study the molecular details in control of transcription in the bacterium. As reported previously, RNA polymerase purified from *H. pylori* was inactive using in vitro transcription assays, indicating unique features and/or requirements in the transcription machinery of *H. pylori*. Expending our expertise from *Escherichia coli* studies, we are attempting to dissect the transcription machinery and to develop an in vitro transcription system for *H. pylori* using different approaches. Our progress in these attempts will be reported and/or discussed.

Virulence Factors and Pathogenesis

Abstract no.: 03.01
Matrix Metalloproteases-7 and -9 Production by *Helicobacter pylori*-Infected Gastric Epithelial Cells Show Differential Gene Participation from *cag* Pathogenicity Island

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Helicobacter pylori infection induces the expression of matrix metalloproteases (MMPs). CagA protein could play a pivotal role in the release of MMPs in gastric epithelial cells. The aim of this study was to identify the mechanism of expression of MMP-9 and -7 in reference to *cag* pathogencity island (*cag*PAI) genes. MMP-9 and -7 productions were measured by reverse transcriptase polymerase chain reaction and gelatin zymography or Western blot. Inducible CagA expression cell lines (a kind gift from Prof. Hatakeyama M. of Hokkaido University) was used. Also, a pair of cagA isogenic mutants with different tyrosine phosphorylation activities was used to check MMP production. *cagE* and *vacA* knockout mutants as well as *cag* PAI absent strain 8822 were used. *CagA*-positive strains are able to induce greater MMP-9 production. It was well correlated with the tyrosine phosphorylation status of the CagA protein. The expression of CagA in AGS cells by inducible system or transient transfection, gastric epithelial cells confirmed that the increased production of MMP-9 is phosphorylation dependent. On the other hand, the expression of MMP-7 is *cag*PAI dependent but CagA phosphorylation independent. Rather than phosphorylation of CagA, either presence of *cagA* and *cagE* or PAI may be important in increased production of MMP-7. The tyrosine phosphorylation of CagA influences the production of MMP-9 by gastric epithelial cells. The presence of *cagE* rather than the phosphorylated CagA seems to be an important factor for the expression of MMP-7. This explains the differential role of *cag*PAI genes' participation in MMPs production by *H. pylori*-infected gastric epithelial cells.

Abstract no.: 03.02
Dendritic Cell Maturation and Release of Cytokines, Chemokines, and Growth Factors in Response to *Helicobacter pylori*: Role of the Urease

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The nature of *Helicobacter pylori* infection is characterized by chronicity of carriage. Bacterial factors, which may play a role in immune escape mechanisms, should be present in every *H. pylori* strain. In this study, the influence of *H. pylori* on maturation and cytokine, chemokine, and growth factor release of dendritic cells (DCs) was investigated with a particular focus on the role of the urease.

Monocyte-derived immature DCs were stimulated for up to 48 hours with *H. pylori* isogenic strains (producing or lacking urease) both intact bacteria and lysates, and recombinant (r) UreA and UreB. Maturation of DCs was determined by flow cytometry. Cell supernatants were tested by the 17-Plex assay of BioRad, which allows for the simultaneous quantitation of 17 cytokines, chemokines, and growth factors. Bioactive TGF-β1 was quantified by conventional enzyme-linked immunosorbent assay.

All stimuli caused maturation of DCs. With the exception of the recombinant proteins, all stimuli induced considerably higher amounts of G-CSF, IL-8, and IL-10 than lipopolysaccharide (LPS) (positive control). In contrast, values of IL-12, IFNγ, and TNFα obtained with LPS were far above those with other stimuli and in particular with recombinant proteins. In contrast to all other stimuli, intact bacteria were shown to induce significant amounts of TGF-β1, which may play a role in gastrointestinal mucosal healing among others down-regulating the Th1 immune response. Furthermore, *H. pylori* urease was shown to up-regulate ($p < .05$) TGF-β1 and MCP-1, which promotes Th2 effector cells.

Thus, the interaction of *H. pylori* with DCs may favor the Th2 immune response.

Abstract no.: 03.03
Activation of Beta-Catenin Signal by *Helicobacter pylori* CagA

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Helicobacter pylori cagA gene encodes a 130–145-kDa CagA protein that is delivered into gastric epithelial cells via the bacterial type IV injection system. Translocated CagA localizes to the inner surface of the plasma membrane where it undergoes tyrosine phosphorylation by Src family kinases. Infection with CagA-producing *H. pylori* is closely associated with the development of gastric adenocarcinoma. Intestinal metaplasia, which is characterized by transdifferentiation of the gastric mucosa to an intestinal phenotype, has been considered to be a precancerous lesion from which gastric adenocarcinoma arises. Recent studies have shown that deregulated beta-catenin signal plays an important role in the pathological transdifferentiation of various cell lineages, including intestinal metaplasia. Also, germline mutation of E-cadherin, which forms a physical complex with beta-catenin, is responsible for the development of hereditary gastric carcinoma. Accordingly, we investigated the possible link between CagA and the E-cadherin/beta-catenin complex. By using the sequential immunoprecipitation-immunoblotting technique, we found that CagA interacts with the cytoplasmic domain of E-cadherin independently of CagA tyrosine phosphorylation. The CagA–E-cadherin interaction inhibits E-cadherin/beta-catenin complex formation, resulting in cytoplasmic/nuclear translocation of beta-catenin. Nuclear accumulated beta-catenin then transactivates beta-catenin–dependent genes that include intestinal-specific transcription factor cdx1. These results raise the possibility that deregulated activation of beta-catenin signal by CagA aberrantly induces *Cdx1*-dependent genes, which mediate the development of intestinal metaplasia, a premalignant gastric mucosal lesion associated with *H. pylori* infection.

Abstract no.: 03.04
Prevalence of Virulent *Helicobacter pylori* Strains in Patients with Ischemic Cerebrovascular Disease: A Multicenter Study

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Background and Aims. Previous studies suggested an association between ischemic cerebrovascular disease and *Helicobacter pylori* infection, in particular CagA-positive strains. Available data are contrasting and come from monocentric studies from referral tertiary centers. The aim of the present study was to assess the prevalence of CagA-positive *H. pylori* strains in patients with ischemic cerebrovascular infection with respect to controls without evidence of atherosclerotic-related diseases.

Methods. A total of 106 consecutive patients (age 76.4 ± 8 years; men 50%) with well-documented history of ischemic cerebrovascular disease and 97 sex-age (age 76.4 ± 8 years; men 45%) and social background-matched controls without relevant vascular diseases. Subjects come from five different regions of Italy. Risk factors for ischemic cerebrovascular disease (familiar history, arterial hypertension, smoking, diabetes mellitus, dyslipidemia, and obesity) were assessed in all subjects. *H. pylori* infection was assessed by ^{13}C-urea breath test (Altana, Milano, Italy). A serological assay for specific IgG against CagA was also performed (Radim, Pomezia, Italy).

Results. Prevalence of active *H. pylori* infection higher in cases (63%) with respect to controls (54%); however, without reaching statistical significance ($p = .2$, OR = 1.21, 95% CI = 0.85–2.61). A significant association was found between patients and controls as concerning Cag-A positivity (41.5% versus 17.5%; $p < .001$, OR = 1.65, 95%CI = 1.74–6.40).

Conclusions. This is the first study assessing the prevalence of active *H. pylori* infection and CagA-positive strains in the setting of the general population. Our findings suggest that CagA-positive, more cytotoxic strains of the bacterium are significantly associated to ischemic stroke.

Abstract no.: 03.05
In Vitro Study of the Role of *Helicobacter pylori* Strains Involved in Low-Grade Gastric Mucosa-Associated Tissue (MALT) Lymphoma in the Proliferation of Lymphocytes

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Helicobacter pylori is involved in the pathogenesis of gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Hussell et al. showed that *H. pylori* can induce in vitro a proliferation of cells obtained from gastric lymphoma biopsies. However, Gerhard et al. provided evidence for an inhibition of lymphocyte proliferation by a secreted *H. pylori* protein. The aim of our study was to test the in vitro proliferation of lymphocytes from different origins in the presence of different *H. pylori* gastric MALT lymphoma strains.

Lymphocytes from peripheral blood mononuclear cells (PBMC) or tonsil cells were isolated by density-gradient centrifugation and cultured in complete RPMI 1640 with 10% fetal calf serum. *H. pylori* sonicates or viable bacteria were added to the lymphocytes with or without PHA/IL-2 (cell proliferation assay versus T-lymphocyte proliferation inhibition assay). Lymphocyte proliferation was determined by BrdU incorporation measured by flow cytometry.

We did not obtain a significant lymphocyte proliferation in the presence of viable *H. pylori* bacteria or sonicates alone. However, all *H. pylori* MALT strains were able to significantly inhibit T-lymphocyte proliferation. This antiproliferative effect was not obtained with culture supernatants nor with other bacteria (*Escherichia coli* and *Campylobacter jejuni*) used as controls. It was also abolished after tryspin treatment of the sonicates.

In conclusion, lymphocytes from PBMC of nongastric MALT lymphoma patients are not able to respond to *H. pylori* MALT strain antigenic stimuli in contrast to lymphocytes obtained from

gastric lymphoma biopsies (Hussel et al.). As described by Gerhard et al., *H. pylori* gastric MALT lymphoma probably harbors an antiproliferative protein.

Abstract no.: 03.06
Helicobacter pylori Induces Gastric Epithelial Cell Invasion in A c-Met and *cag* PAI-Dependent Manner

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Numerous studies have shown that *Helicobacter pylori* is able to interact with gastric epithelial cells, activating signaling pathways, modifying host cellular functions, and inducing cell phenotypes important for carcinogenesis. One of the less explored cell phenotypes induced by *H. pylori* is cellular invasion, and little is known about the mechanisms involved in this process.

Our aim was to investigate the role of *H. pylori* on gastric epithelial cancer cell invasion, and the mechanisms underlying this process. We also examined whether there were differences between strains in their ability to stimulate cell invasion, especially to assess the role of *cag* pathogenicity island (*cag* PAI), CagA, and VacA in cell invasion.

We found that *H. pylori* induced in vitro AGS cell invasion in two well-established invasion assays, collagen type I gels, and Matrigel filters. *H. pylori*-mediated cell invasion was blocked by the c-Met receptor inhibitor NK4, and by silencing c-Met expression with small interference RNA. Supernatants of cells cultured with *H. pylori* showed increased matrix metalloproteinase (MMP)-2 and MMP-9 activity, which was also suppressed by silencing the c-Met receptor. Studies with different *H. pylori* strains revealed that cell invasion, c-Met tyrosine phosphorylation, and increased MMP-2 and MMP-9 activity, were all dependent on the presence of an intact *cag* PAI.

Our findings suggest that *H. pylori* strains with an intact *cag* PAI activate c-Met and induce MMP-2 and MMP-9 activity, possibly increasing extracellular matrix degradation and leading to subsequent invasion of cancer cells.

Abstract no.: 03.07
Diversity in the Genomic Plasticity Zone in *Helicobacter pylori* Strains from Patients with Chronic Gastritis, Duodenal Ulcer, and Gastric Cancer

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Background. Comparison of the two genome sequences from *Helicobacter pylori* has shown that approximately 6–7% of the genes present in one strain is absent from the other and vice versa. About half of the strain-specific genes is found in the plasticity zone (PZ).

Objective. To determine the diversity in the PZ in *H. pylori* strains isolated from patient with gastritis (G), duodenal ulcer (DU), and gastric cancer (GC) and determine if there is any relation between the presence or absence of some genes and disease.

Experimental. Microarrays consist of 1,660 genes covering the J99 and 26695 genomes and were performed to 42 *H. pylori* isolates from 10 patients with G, 10 with DU, and 9 with GC. DNA from each strain was labeled with CY3 and hybridized with a mixture of J99 and 26695 labeled with CY5. Log_2 ratio = Cy5/Cy3 $\geq$ 0.5 value indicates presence of the gene. The association between presence or absence of genes and diseases was analyzed using χ^2.

Results. The gene core was found to be 1319, and 341 (20.5%) were strain-specific genes. Among the variable genes, 30 were statistically associated with one of the diseases: gastritis, ulcer, or cancer ($p < .10$). Many of these genes were on the PZ. These genes included unknown function, restriction-modification systems, outer membrane proteins, and *cag* PAI.

Conclusions. There is variability in the gene content within the PZ in *H. pylori* from individuals with different diseases and some of them are significantly associated with G, DU, or GC.

Abstract no.: 03.08
The Level of FHIT Gene Expression versus Cytotoxicity of *Helicobacter pylori* Strains

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Helicobacter pylori infection is the main cause of inflammation of the gastric mucosa and the development of gastric cancer. *H. pylori* displays considerable genetic diversity, including the presence of strains that produce cytotoxins VacA and CagA. One of the markers to evaluate the risk of gastric cancer is assessment of the fragile histidine triad (FHIT) proapoptotic protein. Inactivation of

the *FHIT* gene is observed in most human neoplasms and many precancerous conditions.

The aim of this study was to evaluate FHIT protein gene expression in the gastric mucosa of patients with symptoms of nonulcer dyspepsia (below 60 years of age), in correlation to the genotype of *H. pylori* strains *cagA* and *vacA*, as compared to the control group without coexisting infection.

The presence of *H. pylori cagA* and *vacA* genes was determined in bacterial DNA samples isolated from 25 patients (50 bioptates), with polymerase chain reaction method, using primers specific for both genes and strain types (s1, s2, m1, m2). The level of FHIT mRNA was established as a ratio to glyceraldehyde-3-phosphate dehydrogenase mRNA with real time reverse transcriptase-PCR method in 100 bioptates, exactly distributed from the antrum and the corpus.

The level of FHIT mRNA was found to be lower in all *H. pylori*-infected patients. A tendency of greater decrease of FHIT mRNA level for gastric mucosa of patients infected with *vacA*+ genotype (s1/m1) and *cagA*-negative *H. pylori* strains than those with *cagA*-positive strains was observed. The obtained results may be valuable in diagnosis and predicting the risk of gastric cancer development.

Abstract no.: 03.09
Adhesin-Receptor Interactions Involved in Adherence of *Helicobacter pylori* to Different Topographical Regions of the Human Stomach

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Background. Infection of the human stomach by *Helicobacter pylori* usually occurs in two main topograpical regions: the antrum and the fundus. Although the distribution of *H. pylori* is known to be influenced by acid, little work has been carried out to determine whether regional variation in the expression of cell-surface receptors also has a role to play; which was the aim of this study.
Methods. Both immunohistochemistry and binding assays (using *H. pylori* mutants lacking the adhesins BabA, SabA, AlpA, AlpB, OipA, and HopZ) were used to determine the presence of *H. pylori* receptors in biopsies from both the antrum and fundus of 10 patients with inflamed stomachs.
Results. Binding to BabA was reduced in all patients and SabA in two patients. Binding to AlpA and OipA was unaffected. Binding to AlpB was reduced in all patients and to HopZ in three patients. No differences were seen between the fundus or the antrum.
Discussion. The results suggest that the same receptors necessary for *H. pylori* adhesion are present in both the antral and fundal regions of inflamed human stomachs. Both BabA and AlpB made significant contribution to binding in all patients. SabA and HopZ was important in two and three patients, respectively. AlpA and Oip A made no contribution to binding.

Abstract no.: 03.10
Helicobacter pylori BabA Expression, Gastric Mucosal Injury, and Clinical Outcome

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Background. BabA, the blood group antigen-binding adhesion, has been proposed to play a role in disease pathogenesis. This hypothesis is based on the functional BabA status as determined by polymerase chain reaction (PCR) analysis to distinguish functional *babA2* genes from nonfunctional *babA1* genes.
Methods. We compared the ability of published PCR-based methods to assess BabA status with BabA immunoblotting and Lewis b (Le^b)-binding activity assays. We also used immunoblotting to examine the relationship between clinical presentation and levels of BabA expression.
Results. Immunoblotting and Le^b binding assays for 80 strains revealed three levels of BabA expression: BabA-high producers (BabA-H) with Le^b-binding activity, BabA-low producers (BabA-L) without Le^b-binding activity, and BabA-negative. BabA-negative strains lacked the *babA* gene. PCR methods to determine BabA status yielded poor results. *babA1* Sequences were never detected. BabA expression was examined in 250 strains from Western countries and 270 from East Asia. The results failed to confirm any relationship between triple-positive status (*cagA*-positive/*vacA* s1/BabA-H) and clinical outcome. BabA-negative strains were typically *cagA*-negative/*vacA* s2 and were associated with gastritis. BabA-L strains exhibited a higher level of mucosal injury and were more frequently associated with duodenal ulcer and gastric cancer than the other groups.
Conclusions. Information gained from currently used PCR-based methods must be interpreted with caution. Le^b-binding activity does not accurately reflect the severity of mucosal damage or the clinical outcome. Quantitation of BabA expression revealed that Le^b-nonbinding BabA-L strains are associated CagA and with higher levels of mucosal injury and clinical outcome.

Abstract no.: 03.11
Relationship of HP-Urease Enzyme with Oxidative Burst and T-cell Migration

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Introduction. In *Helicobacter pylori* gastritis, neutrophil activation plays a central role in the pathogenesis of the disease, and the immune response in the control of infection outcome. Urease is a major component of *H. pylori* surface proteins, and its participation in oxidative stress and inmune response intensity is unknown.
Objectives. To investigate the ability of *H. pylori* urease enzyme on oxidative burst and T-cell migration toward the gastric mucosa.

Materials and Methods. AGS were cultured with two *H. pylori* strains (10^7–10^8 CFU/mL/24 hours), one of them without the *ureC* gene ($HP_{not\ ureC}$).

Lymphocytes and polymorphonuclear leukocyte (PMNL) erythrocytes were separated on Ficoll-Hypaque gradient and PMNLs recuperated after erythrocyte lysis.

PMNLs were incubated with supernatants of AGS/*H. pylori* cultures 90′/37 °C /5% CO_2.

Oxidative stress was assessed with H_2-DCF-DA and MitoSOX. Analyses were performed by flow cytometry and confocal microscopy. For chemotaxis assays, lymphocytes and supernatants were placed in the upper and lower wells, respectively, separated by cellulose nitrate filters (5 µm pore). After 120′ incubation 37 °C/5%CO_2, chemotactic index was calculated as cells migrating toward supernatants or toward medium.

Results. Reactive oxygen species (ROS) synthesis in PMNLs was dose and urease dependent (32% *H. pylori* and 13.5% $H. pylori_{not\ ureC}$ with respect to control) but in AGS, a 1.3-fold increase was observed with all of the strains.

Conclusions. Urease is involved in T-cell recruitment and in oxidative stress observed in mucosa, increasing the ROS synthesis in PMNLs but not in epithelial cells.

	Control	$H. pylori_{not\ ureC}$ 10^7	$H. pylori_{not\ ureC}$ 10^8	*H. pylori* 10^7	*H. pylori* 10^8	FMLP
CI	1	0.64	1.75	1.25	2.61	3.53

FMLP: positive control.

Abstract no.: 03.12
Lack of Association between *dupA*-positive *Helicobacter pylori* Strains and Duodenal Ulcer/ Gastric Carcinoma in Brazilian Patients

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Recently, Lu et al. (2005) described a new putative *Helicobacter pylori* virulence marker (*dupA*) associated with an increased risk for duodenal ulcer (DU) and reduced risk for gastric cancer (GC). We investigated the presence of *cagA* and *dupA* (*jhp0917–0918*) in 482 *H. pylori* strains from Brazilian children (34 DU, 97 gastritis) and adults (126 DU, 144 gastritis, 81 GC). We constructed test on two sets of primers to test again *jhp0917–0918*–negative strains. PCR products from 89 strains were sequenced in order to detect the insertion of C or T (after position 1385) in the *jhp0917* 3′ region that characterizes *dupA*. *jhp0917–jhp0918* Were present in 445 (92.3%) and absent in 29 (6.0%) strains. Because an insertion of T or C was observed in 96.6% of the *jhp0917–jhp0918* positive strains, the presence of the two genes was considered to be *dupA* positive. Strains with only one gene were not included in the analysis. All samples from children with and without DU were *dupA* positive. No association was observed among the strains from adults with gastritis (92.3%), DU (87.3%), and GC (87.6%). Conversely, *cagA*-positive status was independently associated with DU (in adults and children) and with GC in logistic analysis. When children and adults were compared, the presence of *dupA* was significant higher in children, even when only the group of gastritis was analyzed (p = .01). In conclusion, *dupA* is highly frequent and it is not associated with GC or DU in both Brazilian adults and children, which points to regional differences in the distribution of *dupA*.

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Abstract no.: 03.13
Stability and Variability of *cagA* and its Correlation with Disease Outcome

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CagA of *Helicobacter pylori* has been associated with peptic ulcer disease and gastric cancer. CagA can be phosphorylated on tyrosine residues within EPIYA motifs, leading to cell spreading and elongation, a phenomenon termed hummingbird phenotype (HBF). In *H. pylori* strains, the number of phosphorylation sites (PHS) in CagA varies and has been associated with an increase in the extent of HBF. We investigated whether the encoding region of the EPIYA motifs is identical in isolates from different parts of an individual's stomach using isolates from fundus, corpus, and antrum of four patients. Pairs of *H. pylori* strains obtained between 7 and 10 years apart from seven other patients were used to determine the stability of the region through time. We also investigated whether the number of PHS in CagA correlated with disease outcome in 49 Mexican patients with differing gastrointestinal conditions. The *cagA* 3′ region was amplified by polymerase chain reaction, and results were analyzed using agar gel electrophoresis. Sequencing of 10 representative strains from the Mexican patients corroborated a correlation between higher molecular weight bands in gel electrophoresis and the presence of additional EPIYA motifs. The *cagA* 3′ region was identical in *H. pylori* strains isolated from different gastric regions studied, and over time. There was no correlation between the number of PHS in the translated CagA protein and severity of patient's gastrointestinal condition. We conclude that the number of PHS is stable in the predominant isolates over the time period and in the locations tested, but were not associated with clinical outcome.

Abstract no.: 03.14
Characterization of the Number and Type of Repeating EPIYA Phosphorylation Motifs in the Carboxyl Terminus of CagA protein in *Helicobacter pylori* Clinical Isolates

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We aimed to map the EPIYA tyrosine phosphorylation motifs (TPMs) *A*:EPIYAKVNK, *B*:EPIYAQVAKK, and *C*:EPIYATIDDLG in CagA protein, which have been proposed to enhance *cagA*-dependent pathogenicity. Sixty-five *Helicobacter pylori* clinical strains isolated from adults with nonulcer dyspepsia (n = 13), esophagitis (n = 12), gastric ulcer (n = 11), and duodenal ulcer (n = 29) were analyzed. In the 48 cagA-positive strains, the 3′ variable region of *cagA* gene was amplified and sequenced and the EPIYA motifs were mapped in the deduced protein sequences. *H. pylori* colonization and the associated gastritis were evaluated by the modified Sydney system and statistical analysis performed by χ^2 test and Fisher's exact test.

The majority of strains harbored the *ABC* (54.5%) and the *ABCC* combination of TPMs (13.6%). Only four strains were found to harbor additional TPM in the *ABCCC* (n = 3) or *ABABC* (n = 1) combinations. Eighty-five percent of strains isolated from gastroduodenal ulcers harbored *ABC* or *ABCC* combinations of TPMs. EPIYA presence in the CagA protein was correlated significantly with the development of gastroduodenal ulcer ($\chi^2 = 11.617$, $p = .0007$) and in particular with the presence of duodenal ulcer ($p = .0016$). There was significant positive association with the severity of chronic inflammatory infiltration ($p = .039$) and the activity of chronic gastritis ($p = .013$) in the antrum, but not with higher levels of *H. pylori* colonization ($p = .136$). In conclusion, the severity of chronic inflammatory infiltration and the activity of chronic gastritis developed in the antrum of *H. pylori*-positive patients may be associated with the presence of EPIYA TPMs in the CagA protein, irrespective of the levels of *H. pylori* colonization in the gastric mucosa.

Abstract no.: 03.15
Status of *dupA* Gene in Iranian *Helicobacter pylori* Isolates Recovered from Patients with Gastric Cancer

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Introduction. *jhp0917* and *jhp0918* genes are recently identified in the plasticity region, which form one continuous gene designated as *dupA* (duodenal ulcer promoting gene A). *dupA* Is identified as a novel marker of *Helicobacter pylori* associated with an increased risk for duodenal ulcer (DU) and reduced risk for gastric atrophy and cancer. We aimed to determine the prevalence of *dupA* gene in Iranian strains, with concentration on its relationship with histopathologic indices.

Methods. One hundred cases including 35 gastric cancer (GC), 48 nonulcer dyspepsia (NUD), 8 DU, and 9 gastric ulcer (GU) cases were studied. The amplification of *jhp0917* and *jhp0918* genes was performed and the *dupA* genotype was determined. In addition, histologic indices, including *H. pylori* load, atrophy, intestinal metaplasia, and lymphoid follicles were studied.

Results. The *dupA* gene was detected in 44% of Iranian *H. pylori* isolates (4% of isolates were positive for *jhp0917* gene but negative for *jhp0918*). More than half of the isolates from cardia GC patients (55%) and 33% of noncardia GC cases possessed the *dupA* gene, whereas 50% of DU, 41.7% of NUD, and 22.2% of GU cases were *dupA* positive. Among cancer patients, 58% of cases with atrophy and 52.6% of cases with intestinal metaplasia were *dupA* negative. Although the presence of the *dupA* gene was associated with reduced intestinal metaplasia and atrophy, this association was not statistically significant. There was no association with other pathologic indices.

Conclusion. Our preliminary findings indicate that the *dupA* gene can be informative of DU development and reduced risk of atrophy and intestinal metaplasia. However, further studies are required.

Abstract no.: 03.16
Study of the Serum Antibody Response against the Vacuolating Cytotoxin (VacA) from *Helicobacter pylori* in a Mexican Population

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VacA activity differs among isolates as a result of polymorphism in its gene. Its p58 subunit may be type m1 or m2. Type m1 binds more extensively to cells and has been associated more closely with gastric adenocarcinoma than type m2. We aimed to study the antibody response against p58 in a Mexican population.
Methods. Serum and gastric biopsies were obtained from 147 infected individuals. These included 92 cases with chronic gastritis only, 34 with precancerous lesions (intestinal metaplasia, atrophy, or dysplasia), 19 cases with duodenal ulcer, and 2 with gastric cancer. Twenty-seven noninfected individuals were used as controls. Strains were *vacA* genotyped by polymerase chain reaction and recombinant m1 and m2 p58 subunits were used in enzyme-linked immunosorbent assay (ELISA) for serology.
Results. Of 147 infected individuals, 104 were seropositive for VacA; sera from 65 of these recognized both m1 and m2 antigens and sera from 39 recognized one only. From the 39, 37 (95%) had ELISA results that correctly identified the genotype found in isolates (p = .008 Fischer Exact Test). Intensity of the IgG response was higher for m1 than m2 ($p < .05$). Levels of p58 antibodies in precancerous lesions were significantly higher than for duodenal ulcer ($p < .01$).
Conclusion. Mixed serological response to both m1 and m2 antigens is frequent. The m1 toxin induces higher levels of antibodies than m2. High antibody response is significantly associated with precancerous lesions. VacA serotyping is accurate when there is a serological response to only one toxin type.

Abstract no.: 03.17
Increased Amidated Gastrin-17 (PGL) Systemic Levels and Gastric Epithelial Cell Proliferation (GECP) in Dyspeptic Patients with CagA-Positive (CagA+) *Helicobacter pylori* Infection

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Background and Aim. High gastrin systemic levels and augmented gastric epithelial cell proliferation (GECP) are involved in gastric carcinogenesis. Infection with CagA-positive *Helicobacter pylori* is associated with an increased risk of gastric cancer (GC) too. We aimed to verify the possible influence of the CagA status on both these parameters.
Methods. Blood samples and antral gastric biopsies were taken from 116 dyspeptic patients. We determined the *H. pylori* status by histology, serology [enzyme-linked immunosorbent assay (ELISA)] and urease rapid test on biopsies, anti-CagA antibodies by Western blotting, basal amidated gastrin-17 (PGL) by ELISA and epithelial proliferation by anti-proliferating cell nuclear antigen immunostaining as percentage of labeled cells in gastric pits.
Results. Sixty-nine patients (59.4%) were infected and anti-CagA antibodies were detected in 51 of them (73.9%). PGL in patients infected with CagA-positive strains (8.5 ± 3.1 pmol/L) were significantly higher than in patients infected with CagA-negative strains (4.6 ± 1.5 pmol/L) (p = .05) and in uninfected patients (4.8 ± 2.1 pmol/L) (p = .05). Proliferation scores in patients infected by CagA-positive *H. pylori* were also higher in than in CagA-infected and in uninfected patients ($p < .001$, χ^2 test for linear trend).
Conclusions. In *H. pylori*-infected patients, both hypergastrinemia and increased gastric cell proliferation seem to be related to the CagA status rather than to the infection itself. Our results suggest that the increased GC risk run by CagA-positive *H. pylori*-infected individuals could be partially attributed to an up-regulation of growth factors such as gastrin.
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Abstract no.: 03.18
Detection and Quantification of *Helicobacter pylori cagA* Gene in Gastric Biopsies of Chilean Patients by Real-Time PCR

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Background. The *cagA* gene is one of most studied in *Helicobacter pylori* because of its association with atrophic gastritis, intestinal metaplasia, and gastric cancer. The latter, being the second most common cause of death in the world.

In this work, we have used real-time polymerase chain reaction (PCR) technology because of its higher sensitivity and specificity for the analysis of gastric biopsy samples.

The aim of this work was to detect and quantify *cagA* on gastric biopsies from Chilean patients with diverse histopathologies (nonatrophic chronic gastritis, atrophic gastritis, intestinal metaplasia, neoplasia, and gastric cancer).
Methods. We analyzed 88 gastric biopsy samples (one from the antrum and one from the corpus, from 44 patients) positive for the 16Sr RNA gene of *H. pylori*, then the *cagA* gene was detected and quantified by real-time PCR.
Results. *cagA* Was detected in greater percentage, 83% (29/35), in the most aggressive lesion (gastric atrophy, metaplasia, neoplasia, and gastric cancer) when compared with nonatrophic chronic gastritis, 56% (5/9). On the other hand, *cagA* was detected equally in the antrum and corpus. These results also suggest an assosiation between the severity of the gastric injury and the *cagA* gene quantity. That is to say, *cagA*-positive bacteria are more abundant in severe lesions than they are in less severe lesions.

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Abstract no.: 03.19
Distribution and Allelic Diversity of a Novel Peptic Ulcer Marker Candidate in Different Geographical Regions

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Background. *jhp*0870 Was correlated with peptic ulcer disease (PUD) in Portuguese children, and was strongly associated with the more virulent genotypes, whereas its 90% homologue, *jhp*0649, was strongly associated with gastritis and the less virulent genotypes. Both code putative outer membrane proteins. Allelic variation was observed in the midregion of these genes.
Aims. Study prevalence and allelic diversity of both genes in *H. pylori* strains from different countries.
Materials and Methods. Two hundred ninety-one *H. pylori* strains from PUD, nonulcer dyspepsia (NUD) or gastric adenocarcinoma (GC) (Portugal n = 115, Brazil n = 57, France n = 50, USA n = 10, Colombia n = 10, Japan n = 10, Korea n = 10; England n = 7, Sweden n = 7, Norway n = 4, Burkina-Faso n = 11) were genotyped by polymerase chain reaction and sequencing.
Results. Both genes presented heterogeneous distribution, being the major differences in Asian strains (90% for *jhp*0870, 15% for *jhp*0649). *jhp*0870 Correlated with PUD compared to NUD in Brazil, Colombia, and Korea. It showed high prevalence among Norwegian and English PUD strains but not in Swedish ones. It has no difference between PUD and NUD for Portuguese, French, and USA strains and is present in all Japanese strains. It is significantly lower in Portuguese and Brazilian GC strains than overall prevalence in these countries. *jhp*0649 Associated with GC in Portugal and presented low prevalence in Colombian, Japanese, and Korean PUD strains.

Each gene presented predominant but distinct allelic variant.
Conclusions. Both genes displayed a varied worldwide distribution. None constitutes a universal disease-specific marker. *jhp*0870 Tends to be more associated with PUD and *jhp*0649 to GC. Allelic conservation observed in a polymorphic genetic region suggests its importance for the function of proteins coded by these genes.

Abstract no.: 03.20
Prevalence of Virulence-Associated Genes among *Helicobacter pylori* Isolated from a Chilean Population and Its Relationship with Gastric Mucosa Lesions

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Background. *Helicobacter pylori* induces an inflammatory response in the stomach that persists for decades. The biological costs for patients include an increased risk for peptic ulceration and gastric carcinoma. The identification of specific strains by genetic tools that stimulate particular diseases is the main goal for many laboratories. Moreover, the identification of a relationship between virulence markers and geographical areas is also crucial. The aim of this work was to evaluate the prevalence of selected virulence genes *cagA*, *vacA* (alleles s1a, s1b, s2, m1, and m2), *iceA* (alleles 1 y 2), *babA2*, and its relationship to a specific gastric lesion intensity.
Methods. Two hundred sixty biopsy samples (50% from the antrum and 50% from the corpus) were studied. Samples were analyzed by conventional polymerase chain reaction using specific primers that included both a 16S-23S rDNA hypervariable region and ureC markers for species identification.
Results. Forty-eight percent and 57.7% of the samples contain *H. pylori* as detected by 16S-23S rDNA and ureC primers, respectively. Virulence genes identified were as follows: *cagA* 32.7%, *vacAs1a* 40.7%, *vacAs1b* 20.7%, *vacAs2* 32.7%, *vacAm1* 42.0%, *vacAm2* 44.7%, *iceA1* 26.0%, *iceA2* 67.7%, and *babA2* 5.3%, respectively. There was no significant difference in the positive rates of gene detection and the intensity of the lesion in patients. In addition, no differences were also observed among genetic markers between the antrum and the corpus isolates in the same patient.
Conclusion. In Chilean patients, no correlationship between virulence markers of the clinical isolates of *H. pylori* and gastric lesions intensity was observed.

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Abstract no.: 03.21
Relationship between *Helicobacter pylori* CagA and VacA Serological Status and Bacterial Density on Gastric Mucosa

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Aim. To evaluate the effects of CagA and VacA seropositivity on *Helicobacter pylori* bacterial density in bioptic samples of gastric mucosa.
Methods. Study was carried out on 207 patients undergoing upper endoscopy. All patients were *H. pylori* positive based on results of histology and serology. To all patients, serum antibodies to p 120 (CagA) and p 95 (VacA) proteins of bacteria *H. pylori* were assessed by using Western blot method. Histological data were analyzed according to updated Sydney classification with special attention to the grade of *H. pylori* colonization. Statistical analysis was performed using χ^2 test.
Results. the study population consisted of 91.3% (189/207) CagA antibody positive and 75.8% (157/207) VacA antibody-positive patients. The distributions of CagA- and VacA-positive and -negative patients according to *H. pylori* bacterial load grade are presented in Table 1.
Conclusions. CagA and VacA seropositivity have no influence on bacterial load grade in the gastric mucosa.

Table 1 CagA and VacA serological status and bacterial density

	Corpus			Antrum		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Garde 3
CagA+	108	50	31	66	69	54
CagA–	13	2	3	9	4	5
VacA+	92	40	25	55	62	40
VacA–	30	12	8	20	11	19

No statistically significant difference in grades of bacterial load was found between CagA-positive and CagA-negative patients in the corpus and antrum of the gastric mucosa ($\chi^2 = 2.180$, d.f. 2, $p < 1.0$; $\chi^2 = 1.986$, d.f. 2, $p < 1.0$). The same results were obtained for VacA-postive and VacA-negative patients ($\chi^2 = 0.0515$, d.f. 2, $p < 1.0$; $\chi^2 = 5.634$, d.f. 2, $p < 1.0$).

Abstract no.: 03.22
Vacuolating Activity of Different *Helicobacter pylori* Colonies Isolated from the Same Dyspeptic Patients with Peptic Ulcer (PU) or with Chronic Gastritis Only

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Background. Infection by vacuolating cytotoxic *Helicobacter pylori* strains (VacA+) increases the risk of peptic ulcer (PU). As infected patients may simultaneously harbor various *H. pylori* genotypes, we tested four to eight different *H. pylori* colonies per patient to better understand the pathogenic role of VacA.
Material and Methods. We examined 78 *H. pylori* strains, 38 from 11 patients with PU and 40 from 11 patients without ulceration (WU). Forty-eight–hour agitated broth cultures were centrifuged and sterilized by filtration. Filtrates were added to Vero, HeLa, and Chinese hamster ovary cells in vitro at dilutions ranging from 1 : 2 to 1 : 32, in triplicate. Strains were considered VacA positive if ≥ 30% of cells of whatever line were vacuolated after 24 and 48 hours of incubation. The "s" and the "m" subtypes of the *vacA* gene were determined by polymerase chain reaction.
Results. In only 15 of the 22 patients that all strains from the same patients were either cytotoxic or noncytotoxic; 25 of 38 strains from PU patients were VacA positive (65.7%), versus 15 of 40 strains from WU patients (37.5%) ($p = .013$; OR = 3.21, 95% CI = 1.15 to 9.06). Almost all VacA positive strains were s1/m1 *vacA* subtype.
Conclusions. A fair proportion of patients harbors either VacA-positive and VacA-negative strains at the same time. Oscillation in the predominance of VacA-positive and VacA-negative bacterial population may account for spontaneous healing and recrudescence of PU disease.
Acknowledgements. This study was partly funded by the Siena University grant PAR 2004, "*Helicobacter pylori* infection, host's aplotypes of inflammatory cytokines and risk of ischemic heart disease".

Abstract no.: 03.23
Cyclooxygenase-2 Expression in *Helicobacter pylori*-Infected Early Gastric Carcinoma

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Introduction. Previous studies demonstrate that increased cyclooxygenase-2 (cox-2) expression is observed in both *Helicobacter pylori*-related gastritis and gastric carcinoma. Little is known about the cox-2 expression in *H. pylori*-infected gastric carcinoma.
Aim. We studied the expression of cox-2 in *H. pylori*-infected early gastric cancer tissue after being successfully treated with endoscopic mucosal resection and their paired adjacent mucosa, as well as their follow-up endoscopically biopsied mucosa after *H. pylori* eradication.
Method. The expression of cox-2 in 20 patients with *H. pylori*-infected early gastric carcinoma (15 men and 5 women; mean age, 63.8 years) and their follow-up biopsy (mean follow-up period, 27.4 months) was assessed by immunohistochemical stain. Immunoreactive score was calculated by multiplication of the grade determined by the percentage of positive cells and the staining intensity (scale, 0–9).
Results. Slightly increased expression of cox-2 was found in cancer tissue compared to their respective paired *H. pylori*-infected normal mucosa (4.5 ± 3.7, 3.2 ± 1.9), but statistically not significant ($p = .07$). After *H. pylori* eradication, the cox-2 expression was markedly decreased (1.5 ± 1.3, $p < .05$).
Conclusion. Cox-2 overexpression may contribute to an early event of *H. pylori* infected gastric carcinoma.

Abstract no.: 03.24
Distribution of Virulence Marker of *Helicobacter pylori* Patients with Functional Dyspepsia in Pakistan

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Aim. To determine the distribution of virulence markers of *Helicobacter pylori cagA, vacA,* and its allele types in patients with dyspepsia.
Methodology. Endoscopic-negative functional dyspepsia patients were enrolled from January to December 2005. Gastric antral biopsies were obtained for rapid urease test, histopathology, culture, and polymerase chain reaction (PCR) for *H. pylori* virulence markers *cagA, vacA,* and its allele types. PCR for *cag A* and *vacA* alleles were performed using primers previously described.
Results. Of 94 patients, 64 (68%) were men, age range 18–70 years and mean age 40 ± 11.3 years. Epigastric pain syndrome was present in 81 (86%) and postprandial stress syndrome in 13 (14%). Esophagogastroduodenoscopy showed hyperemia in all 94 (100%). Rapid urease test was positive in 89 (95%). Histopathology was only performed in 53 (56%) and it showed *H. pylori*-associated moderate gastritis in 36 (68%) and mild gastritis in 12 (23%) whereas nonspecific gastritis in 5 (9%). The PCR for *cagA* was positive in 39 (41%) and negative in 55 (59%). The *vacA* allelic types were *s1a m1* 41 (44%), *s1a m2* 22 (23%), *s1b m1* 8 (9%), and *s1b m2* 19 (20%); however, it could not be obtained in four

isolates. *CagA s1a m1* was positive in 16 (17%), *cagA s1a m2* 11 (12%), *cagA S1b m1* in 3 (3%), and *cagA S1b m2* in 8 (9%).
Conclusion. In functional dyspeptic *H. pylori,* infection was frequently with *cagA*-negative strains. However, the dominant virulence pattern in these isolates was cagA-positive vacA S1a m1 allelic type.

Abstract no.: 03.25
Virulence Genotypes of *Helicobacter pylori* in Palermo, Italy

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Aims. To determine the distribution and/or the functional status of *s1-vacA, cagA, oipA, babA2, sabA,* and *hopQ* genotypes of *Helicobacter pylori* strains isolated from patients with gastroduodenal diseases in Palermo, Italy, and to explore the association between the different genes and their usefulness for predicting the clinical outcome of infections.
Methods. Forty-three strains of *H. pylori* were isolated from gastric biopsies of 24 patients with gastritis (16) and/or duodenal ulcer (8); only 28 of these strains were considered for evaluation; 15 were excluded as considered duplicates after genotyping. The virulence genotypes were determined by polymerase chain reaction.
Results. The *s1-vacA, cagA*$^+$ (all *s1-vacA*), *s1/m1-vacA,* and *s1/m1-vacA/cagA* positive genotypes were detected in 75% (21/28), 64.3% (18/28), 42.9% (12/28), and 32.1% (9/28), respectively, of the *H. pylori* strains. The *oipA* "on" status, exhibited by 31.8 (7/22) of the strains, showed only a weak association with the *cagA* gene (71.4% of the *oipA* "on" strains were *cagA* positive, 35.7% of the *cagA*-positive strains were *oipA* "on"); the *babA* positive and the *sabA* "on" genotypes, present in less than 25% of the strains, were strongly associated with each other and with the *cagA*-positive genotype; the type I *hopQ* allele was predominant, and predominantly (but not always) associated with the *s1-vacA/cagA*-positive genotype: in 29.2% (7/24) of the strains. After amplification, both types I and II *hopQ* products were observed.
Conclusions. Many results are concordant with data in literature. Differences, to be correctly evaluated, might be confirmed by additional isolations.

Abstract no.: 03.26
Functionally Active *Helicobacter pylori* Vacuolating Cytotoxin in *Escherichia coli*

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Background and Aims. Although the expression of VacA toxin in *Escherichia coli* has been attempted, the production of a functionally active recombinant version has been rare. In this study, we produced the active recombinant VacA to gain further insights into the pathological activities of VacA in gastric epithelial cells.
Methods. A soluble form of about 90-kDa VacA s1/m1 genotype fused with 8X histidine tagged at the C terminus was expressed in *E. coli* at low temperature. The antisera was raised in a rabbit by intradermal injections of this recombinant protein.
Results. The recombinant VacA was able to induce vacuolation and apoptosis in AGS cells and HeLa cells. Anti-rVacA antibody reacted with supernatants from *Helicobacter pylori* carrying the s1/m1 *vacA* gene in an enzyme-linked immunosorbent assay and an immunoblot with a 88-kDa protein. Furthermore, the immunoglobulins neutralized the vacuolating activity completely and inhibited cell death induced by both rVacA and supernatant of *H. pylori* in AGS cells.
Conclusion. These data indicate that this recombinant VacA has function and structure similar to those of native VacA, which could be useful in exploring the pathogenic role of VacA.

Epidemiology and Transmission

Abstract no.: 04.01
Environmental and Host Factors Associated with Risk of *Helicobacter pylori* Infection in Brazil

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There are few studies evaluating host genetics as risk factors for *Helicobacter pylori* infection. We investigated associations between functional polymorphisms in genes linked to the innate and adaptative immune response and susceptibility to HP infection in logistic models, adjusting for demographic and environmental factors. We included 541 blood donors; 370 (68.4%) were *H. pylori* positive by enzyme-linked immunosorbent assay. *IL1B*-511C/T, *TNFA*-307G/A, *TLR4*+896A/G and *TLR2*+2251G/A were genotyped by polymerase chain reaction (PCR)-restriction fragment-length polymorphism, *IL1RN* by PCR, *IL1B*-31T/C, and *IL2*-330T/G by PCR-confronting two-pair primer (CTPP) and *TLR5*+1174C/T by allele-specific PCR. All results were confirmed by sequencing. *IL1B*-511 and *IL1B*-31 were in almost complete linkage disequilibrium, whereas the other loci segregated independently. The models were well fitted and in the univariate analysis, age ($p = .001$), gender ($p = .02$), crowding index ($p = .20$), socioeconomic