



THE AGA KHAN UNIVERSITY

eCommons@AKU

Section of Gastroenterology

Department of Medicine

August 2009

Associations of the Plasticity Region of *h. pylori* in Patients with Gastroduodenal Diseases

Javed Yakoob

Aga Khan University, javed.yakoob@aku.edu

Z Abbas

Aga Khan University, zaigham.abbas@aku.edu

Shagufta Naz

Aga Khan University

Shahab Abid

Aga Khan University, shahab.abid@aku.edu

Wasim Jafri

Aga Khan University, wasim.jafri@aku.edu

Follow this and additional works at: http://ecommons.aku.edu/pakistan_fhs_mc_med_gastroenterol



Part of the [Gastroenterology Commons](#)

Recommended Citation

Yakoob, J., Abbas, Z., Naz, S., Abid, S., Jafri, W. (2009). Associations of the Plasticity Region of *h. pylori* in Patients with Gastroduodenal Diseases. *Helicobacter*, 14(4), 376-376.

Available at: http://ecommons.aku.edu/pakistan_fhs_mc_med_gastroenterol/120

HSCs. The analysis revealed that 13 pathways were upregulated and 22 pathways were downregulated by microRNA. Furthermore, mitochondrial integrity based on highly upregulated Bcl-2 and downregulated caspase 3, 9 was confirmed in HSCs and fibrotic livers by immunofluorescence assay, semiquantitative RT-PCR, qRT-PCR, and Western blot. These findings provide in vitro and in vivo evidence that the mitochondrial pathway of apoptosis plays a significant role in the progression of liver fibrogenesis via HSCs activation.

Abstract no.: P05.28
The Prevalence of *H. pylori* Infection in the Patients with Different Gastroesophageal Reflux Disease Groups

E. V. Onuchina* and V. V. Tsucanov†

*Irkutsk State Medical University, Irkutsk, Russia; †Scientific Research Institute of Medical Problems of North, Krasnojarsk, Russia

Aim: To study the prevalence of *Helicobacter pylori* infection in patients with GERD belonging to different age groups.

Material and Methods: A total of 722 patients were investigated: 326 patients older than 60 years and 244 patients under 60 years with GERD; the control group consisted of 152 patients without GERD. The severity of esophagitis was evaluated using the Los Angeles classification. The diagnosis of *H. pylori* was carried out with histologic method and rapid urease test.

Results: *H. pylori* infection in GERD patients is revealed more seldom than in the control group. The chances ratio (CR) of association of *H. pylori* infection and GERD in patients older than 60 years were 0.28 (95%CL: 0.10–0.43), $p = .00001$, in the patients under 60 years it was – 0.59 (95%CL: 0.14–1.03), $p = .00001$. The analysis of clinical forms of GERD revealed that infection was the least in Barrette esophagus (BE) in both age groups. The analysis shows that in young patients the prevalence of *H. pylori* was higher in ERD, in elderly patients – in NERD. In increasing severity of esophagitis in the elderly, decrease of helicobacteriosis spreading was noted, while in young patients the spread of helicobacteriosis increased.

Conclusion: Level of helicobacteriosis not depending on age had distinct reverse connection with the presence of BE in patients of elderly group with severity of GERD.

P06 Molecular Genetics and Genomics, Virulence Factors and Pathogenesis I

Abstract no.: P06.01
Prevalence of *cagA* and *jhp0947* Genes in *H. pylori* Isolates from First-Degree Relatives of Gastric Cancer Patients

S. Massarrat,* F. Siavoshi,† H. Ghadiri,† M. Mirzaei* and M. Zamani†

*Digestive Disease Research Center, Shariati Hospital, Medical Sciences/University of Tehran, Tehran, Iran; †Microbiology Department, Faculty of Sciences, University of Tehran, Tehran, Iran

Introduction: *Helicobacter pylori* gastritis is a dynamic and progressive process. Studies suggest that a combination of different virulence genes determine the severity of inflammation. The aim of this study was to investigate the prevalence of *cagA* and *jhp0947* genes in the first-degree relatives of gastric cancer patients (FDRCPs) and their correlation with different types of gastritis.

Methods: One hundred and forty-three *H. pylori* strains were isolated from antral gastric biopsies of FDRCPs. All of the patients had gastritis. Three types of gastritis according to pathologic findings were: antral-predominant gastritis, corpus-predominant gastritis, and pangastritis. Genomic DNAs from isolates were subjected to PCR-based genotyping of *cagA* and *jhp0947* genes. Primers were designed for amplification of these genes.

Results: The prevalence of the *cagA* and *jhp0947* among 143 *H. pylori* isolates from FDRCPs.

Type of gastritis

Pangastritis	Antral predominant	Corpus predominant	Genotype status
54 (%73)	50 (%78.1)	5 (%100)	<i>CagA</i> positive
38 (%51.4)	35 (%54.7)	1 (%20)	<i>jhp0947</i> positive
74 (%51.7)	64 (%44.7)	5 (%3.6)	Total

Discussion: *cag A*-positive and *jhp0947*-positive *H. pylori* strains were predominant in FDRCPs. However, we could not find any association between genotypes of *H. pylori* strains and different types of gastritis. Although a number of putative *H. pylori* virulence genes have been associated with risks of a clinical outcome, none have clearly been linked to one specific *H. pylori*-related disease. Further studies with more isolates and candidate genes might help to predict the clinical outcome of *H. pylori* infection with certain genotypes.

Abstract no.: P06.02
Prevalence of *cagA* and *jhp0947* Genes in *H. pylori* Isolates from Patients with Gastritis, Gastric Ulcer, and Gastric Cancer

A. Asgharzadeh,* H. Ghadiri,† R. Malekzadeh,‡
 S. Latifi-Navid‡ and A. Sadjadi‡

*Soil Microbiology Department, Soil and Water Research Institute, Tehran, Iran; †Microbiology Department, Faculty of Sciences, University of Tehran, Tehran, Iran; ‡Digestive Disease Research Center, Shariati Hospital, Medical Sciences/University of Tehran, Tehran, Iran

Introduction: *Helicobacter pylori* plays an important role in the development of peptic ulcers, gastric adenocarcinoma, and gastric MALToma. Strains that possess the *cag* PAI/*cagA* are more associated with peptic ulcer and gastric cancer. The *jhp0947* gene, located in the plasticity region of *H. pylori* genome, could report to be associated with an increased risk of both duodenal ulcer and gastric cancer. In this study prevalence of *cagA* and *jhp0947* genes in Iranian *H. pylori* isolates and their relationship with gastrointestinal disorders were evaluated.

Methods: Seventy-five *H. pylori* strains were isolated from antral gastric biopsies. DNA was extracted and primers were designed for amplification of *cagA* and *jhp0947* genes. PCR was performed and the products were electrophoresed.

Results: *cagA*+ was the predominant genotype among *H. pylori* isolates from all three groups of patients: ulcer (%76.2), gastritis (%78.5), and cancer (%40). The *cagA*+, *jhp0947*+ genotype was quite prevalent: ulcer (%59.5), gastritis (%46.4), and cancer (%20).

Discussion: Until now no clear association has been found between host genetics, environmental factors, and *H. pylori* genotype and the gastric diseases. In this study no association was found between *H. pylori* genotypes and type of gastric disease. Further studies on *H. pylori* isolates from different groups of patient are needed to find more candidate genes involved in pathogenicity of *H. pylori*.

Abstract no.: P06.03
***H. pylori* Induces β 3GlcNAc-T5 in Gastric Epithelial Cells Leading to Expression of Sialyl-Lewis X, the Ligand for SabA Adhesin**

N. T. Marcos,* A. Magalhães,* B. Ferreira,*
 M. Oliveira,* N. Mendes,* T. Gilmartin,† S. Head,†
 A. S. Carvalho,* C. Figueiredo,** L. David,**
 F. Santos-Silva** and C. A. Reis**†

*Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal; †The Scripps Research Institute, La Jolla, California, USA; **Medical Faculty of the University of Porto, Porto, Portugal

Helicobacter pylori can produce phenotypic alterations in gastric epithelial cells. Expression of the inflammation-associated sialyl-Lex antigen in the gastric epithelium is induced during persistent *H. pylori* infection, suggesting that *H. pylori* may trigger the host tissue to re-tailor the gastric mucosal glycosylation patterns to a more favorable environment for its adhesion. *H. pylori* has been shown to adhere to glycoconjugates expressed in the gastric mucosa

through bacterial adhesins (BabA, SabA). We evaluated the epithelial cells gene expression in response to *H. pylori* infection. Our results showed that *H. pylori* induced significant alterations in 168 of the 1031 genes tested in a microarray platform. The most virulent *H. pylori* strain led to altered expression of glycosylation-related genes, such as increased expression of β 3GlcNAcT5, a glycosyltransferase involved in the synthesis of Lewis antigens. Further evaluation of a panel of *H. pylori* strains showed that induction of β 3GlcNAcT5 expression was elicited only by the virulent *H. pylori* strains (*cag*PAI+) [1]. β 3GlcNAcT5 overexpression in stably transfected gastric cell lines leads to increased expression of sialyl-Lex antigen and increased adhesion of *H. pylori* [1]. In conclusion, our results show that highly pathogenic *H. pylori* strains induce β 3GlcNAc-T5 and sialyl-Lex expression, the receptor for SabA adhesin, contributing to a successful infection. [1] Marcos NT et al. *J Clinical Investigation* 2008, 118:2325–2336.

Abstract no.: P06.04
FUT2-Null Mice Show Impaired BabA-Mediated Adhesion of *H. pylori* to Gastric Mucosa

A. Magalhães,* J. Gomes,* L. David,** R. Haas,‡
 T. Borén§ and C. Reis**†

*Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal; †Medical Faculty of the University of Porto, Porto, Portugal; ‡Max von Pettenkofer Institute, LMU München, München, Germany; §Department Medical Biochemistry and Biophysics, Umeå University, Umeå, Sweden

Helicobacter pylori adhesion is mediated by glycoconjugates expressed on gastric epithelial cells and constitutes a crucial step in the establishment of a successful infection. The blood group antigen-binding adhesin (BabA) binds to Lewisb and H type-1 structures expressed on gastric mucins, while a sialic-acid binding adhesin (SabA) recognizes sialylated carbohydrates mediating the adherence to inflamed gastric mucosa. Inactivating mutations in human *FUT2* (secretor) gene are associated with reduced susceptibility to *H. pylori* infection. In this study we have used an animal model of non-secretors, *FUT2*-null mice, to evaluate the adhesion of *H. pylori* strains with different adhesins expression profile to the gastric mucosa of *FUT2*-null mice in comparison with the C57Bl/6 *wild-type* mice.

We have demonstrated that *FUT2*-null mice showed marked alteration in gastric mucosa glycosylation, characterized by diminished expression of α (1,2)fucosylated structures as indicated by lectins and antibodies staining. We further analyzed whether these modifications would have a role in *H. pylori* adhesion. *H. pylori* 17875/Leb strain (BabA+), that only expresses a functional BabA adhesin, bound to the foveolar epithelium of *wild-type* mice but no adhesion was observed in *FUT2*-null mice, while 17875*babA1A2* strain (BabA-/SabA+) bound similarly the foveolar epithelium of both mice. The J99 strain (BabA+/SabA+) bound to both mice gastric mucosa, but adhesion levels were decreased in the *FUT2*-null mice. We further evaluated the adhesion of a panel of strains from clinical isolates that were characterized for BabA and SabA expression, showing that BabA-dependent adhesion was impaired in the *FUT2*-null mice, whereas SabA-mediated binding was not affected.

Abstract no.: P06.05
Cloning and Expression of *H. pylori* Type IV Secretion System in *Escherichia coli* Cells by Detection of the Hummingbird Phenotype in Cultured Infected AGS Cells

O. Contreras, J. L. Palacios, E. Bruce, I. Mansilla and A. Venegas

Department of Molecular Genetics and Microbiology, Faculty of Biological Sciences, Pontificia Universidad Catolica de Chile, Santiago, Chile

The *Helicobacter pylori* type IV secretion system (T4SS) is encoded by the 40 kb Cag pathogenicity island (PAI) which contains approximately 30 genes, including *cagA*. This system delivers the bacterial CagA protein into the cytoplasm of the host epithelial cell, promoting chronic infection and late gastric cancer. This T4SS has been characterized previously based on its similarity to the *Agrobacterium tumefaciens* T4SS. However, only a few components of the *H. pylori* T4SS have been identified, and it is thus defined as an incomplete system. To determine if genes located outside the PAI participate in the secretion system, the whole *H. pylori* J99 island was cloned and its functionality evaluated in *Escherichia coli*. Chromosomal fragments were ligated into a pEpiFOS-5 fosmid and transferred to *E. coli* DH5 α . Subsequently, a single clone carrying the entire island was isolated. The *cagA* encoded by PAI presented a deletion and was complemented with a plasmid carrying *cagA* from the strain G27. T4SS functionality was evaluated by inducing the "hummingbird" elongated phenotype of *E. coli*-infected AGS cultured cells. *E. coli* cells carrying the PAI, and complemented with the G27 *cagA* gene, induced this phenotype. The results showed that, after 48-hour infection, 30% of AGS cells displayed the "hummingbird" phenotype compared to 45.5% obtained after *H. pylori* G27 infection and 5% for uninfected control cells. These results suggest that *cagPAI* genes operate in *E. coli* cells and that CagA is translocated and phosphorylated inside AGS cells.

Funded by CTU06 Biomedicina Area 5 and Fondecyt 1085232 grant projects.

Abstract no.: P06.06
***Helicobacter suis* Induces Epithelial Cell Death Both in vivo and in vitro**

B. Flahou,* F. Haesebrouck,* K. D'Herde,† F. Pasmans,* K. Chiers* and R. Ducatelle*

*Department of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; †Department of Basic Medical Sciences, Faculty of Medicine and Health Science, Ghent University, Ghent, Belgium

Helicobacter suis colonizes the stomach of more than 60% of slaughter pigs. Moreover, it is the most prevalent gastric non-*H. pylori* *Helicobacter* species in humans. Recently, this bacterium has been isolated in vitro from the stomach mucosa of slaughter pigs. Little is known about the virulence mechanisms of this and other gastric non-*H. pylori* *Helicobacter* species. Therefore, mice of two strains (BALB/c and C57BL/6) and Mongolian gerbils were inoculated intragastrically with this *Helicobacter* species. Transmission electron microscopy revealed the presence of *H. suis* in close

contact with necrotic gastric epithelial cells, mainly parietal cells. Moreover, *H. suis* colonization was associated with an increased proliferation of mucosal epithelial cells. Both findings suggest a role for *H. suis* in the loss of gastric epithelial cells. In vitro, two gastric epithelial cell lines (human-derived AGS and mouse-derived GSM06 cells) were treated with sonicated *H. suis*. In both cell lines, these whole bacterial cell lysates induced cell death. Fluorescent staining with propidium iodide and antibodies directed against activated caspase-3 showed that both necrosis and apoptosis were present. Pretreatment of whole bacterial cell lysates with heat and trypsin abolished the cell-death-inducing capacity, allocating one or more proteins as the causative agent(s). Further studies should be undertaken to reveal the exact underlying mechanism.

Abstract no.: P06.07
Study of the Lipopolysaccharide of *H. pylori* Gastric MALT Lymphoma-Associated Strains: A Link with Lymphoma Pathogenesis?

P. Lehours,* Z. Cheng,† A. Skoglund,† F. Mégraud* and L. Engstrand†

*INSERM U853, Bordeaux, France; †Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden, and Swedish Institute for Infectious Disease Control, Solna, Sweden, Stockholm, Sweden

The aim of this project was to investigate the Lewis antigen expression in *Helicobacter pylori* gastric MALT lymphoma-associated strains in comparison to chronic gastritis only strains.

Forty MALT strains (19 *cagPAI* (-) and 21 *cagPAI* (+)) and 39 gastritis strains (17 *cagPAI* (-) and 22 *cagPAI* (+)) were included in this study. The LPS for each strain was extracted using hot phenol method and the expression of Lewis X and Lewis Y antigens were investigated using Western Blot. The data were analyzed according to the strains' *cagPAI* status and *vacA* genotype.

Lewis X antigens were identified in 21 MALT strains (52.5%) and Lewis Y in 30 strains (75%). Lewis X antigens were identified in 29 gastritis strains (74.3%) and Lewis Y in 31 strains (79.5%). There was an association between *cagPAI* status and Lewis X expression among MALT strains ($p < .0001$), but not in gastritis strains ($p = .64$).

Considering the disease status, among *cagPAI* (-) strains, the majority of gastritis strains (64.7%) were both Lewis X and Y positive, whereas the majority of MALT strains (63.2%) were Lewis Y positive only: strains expressing solely Lewis Y were associated with MALT (odds ratio = 64.2 (4.9–841.0)). No such association was found in *cagPAI* (+) strains. *vacA* genotypes did not modify the association between Lewis and disease status.

In conclusion, Lewis X antigens in *cagPAI* (+) MALT strains could participate to underbalance their proinflammatory effect. *cagPAI* (-) MALT strains have a particular Lewis antigen profile which could represent an adaptive mechanism to the host response.

Abstract no.: P06.08
The Intermediate Allele of *vacA* and the Vacuolation Induced by s1m2 Genotypes of *H. pylori* Strains

M. Douraghi,* M. Shokrgozar,[†] S. Saberi,* H. Zeraati,[‡] F. Ebrahimzadeh,* M. Esmaeili,* M. Bababeik,* A. Oghalaie* and M. Mohammadi*

*Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran; [†]Department of Cell Bank, Pasteur Institute of Iran, Tehran, Iran; [‡]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Introduction: Several studies have previously demonstrated the s1m2 genotype as the predominant *vacA* genotype among circulating *Helicobacter pylori* strains in Iran. The functional toxicity of such *vacA* genotypes was not however determined. On the other hand, introduction of intermediate region of *vacA* provided new insights into the structural toxicity of *vacA*. The current study aimed to evaluate whether the intermediate region is involved in the toxicity of s1m2 *vacA* genotype strains.

Methods: A total of 109 *H. pylori* single-colony strains were studied. The multiplex polymerase chain reaction (PCR) of s and m *vacA* alleles were followed by i *vacA* PCR. Concentrated culture filtrates (CCF) of all of the studied strains were collected through liquid culture. HeLa cell line was incubated with 1:5 dilution of CCF up to 24 hours. The number of vacuolated cells was determined by inverted light microscopy. The statistical analysis was performed using Mann-Whitney test.

Results: In this study 27.5%, 1.8%, and 29.4% of studied strains were typed as s1i1m1, s1i2m1, and s2i2m2, respectively. Typing of the remaining strains showed that 19.3% were s1i1m2 genotype, whereas 22% were s1i2m2. Having studied the toxicity of s1m2 strains, we showed that number of vacuolated cells incubated with s1i1m2 CCF was significantly greater than that of s1i2m2 strains ($p < .001$).

Conclusion: This study suggested that intermediate allele is informative as toxicity assay and i *vacA* typing is able to classify more toxic types of s1m2 strains. This study confirms the application of such typing in substitution of the time consuming and laborious cytotoxicity assay.

Abstract no.: P06.09
Distinct Presence of CagA-Positive Strains with Higher Number of EPIYA-C Repeats in the Fundus Versus the Antrum of *H. pylori*-Infected Patients

E. Panayotopoulou,* K. Papadakos,* K. Petraki,* B. Martinez-Gonzalez,* S. Michopoulos,[†] G. Mantzaris,[‡] G. Papatheodoridis,[§] A. F. Mentis,* D. N. Sgouras* and A. Archimandritis[§]

*Institut Pasteur Hellenique, Athens, Greece; [†]Gastroenterology Clinic, "Alexandra" Hospital, Athens, Greece; [‡]Gastroenterology Clinic, Athens, Greece; [§]Second Dept of Internal Medicine, Athens University School of Medicine, Athens, Greece

The EPIYA tyrosine phosphorylation sites are important determinants of CagA virulence presenting extensive variability in both the type and the number, especially the EPIYA-C sites, among wild-type *H. pylori* of Western origin.

We have previously identified the occurrence of mixed infections by isogenic *H. pylori* strains with variable number of EPIYA-C repeats within the same patient and we aimed to assess whether they preferentially colonize distinct compartments of the gastric mucosa, namely the gastric antrum and fundus.

CagA and EPIYA status were determined by polymerase chain reaction (PCR) and sequencing in *H. pylori* isolates from 140 paired antral and fundic biopsies from 70 Greek adult patients. Clonal relations between strains were assessed by RAPD-PCR and MLST analysis of the housekeeping genes *atpA*, *efp*, *mutY*, *ppa*, *trpC*, *ureI*, *vacA*, and *yphC*.

In all cases with the exception of one, paired isolates of antral and fundic origin were clonally related. In 59 patients, the same strain was isolated from both the antrum and the fundus (20 cagA-negative, 23 ABC, 11 ABCC, and 5 ABC/ABCC isolates), whereas in 10 patients the fundus was colonized by cagA-positive *H. pylori* harboring more EPIYA-C repeats compared to the corresponding strains from the antrum. In conclusion, with regard to the CagA EPIYA status, the vast majority of individuals were found to be infected by the same *H. pylori* strain. Nevertheless, in approximately 15% of the patients, isogenic strains carrying more EPIYA-C repeats were identified, preferentially colonizing the gastric fundus, possibly reflecting a response to microenvironmental differences in acidity between the two gastric sites.

Abstract no.: P06.10
CagA and VacA Polymorphisms are Associated with Distinct Pathologic Features in *H. pylori*-Infected Adults with Ulcer and Nonulcer Disease

E. G. Panayotopoulou,* K. Papadakos,* K. Petraki,* B. Martinez-Gonzalez,* S. Michopoulos,[†] G. Mantzaris,[‡] G. Papatheodoridis,[§] A. F. Mentis,* D. N. Sgouras* and A. Archimandritis[§]

*Institut Pasteur Hellenique, Athens, Greece; [†]Gastroenterology Clinic "Alexandra" Hospital, Athens, Greece; [‡]Gastroenterology Clinic, "Evangelistos" Hospital, Athens, Greece; [§]Second Department of Internal Medicine, School of Medicine, Athens University, Athens, Greece

CagA and VacA polymorphisms define *Helicobacter pylori* virulence and may predict the development of severe gastric disease. We determined the variability of functional EPIYA tyrosine phosphorylation motifs in CagA and the isotypes for signal, intermediate, and middle regions of VacA in Greek adults with duodenal ($n = 44$) or gastric ($n = 21$) ulcers and nonulcer dyspepsia cases ($n = 79$) and assessed potential associations to the severity of histopathology.

EPIYA motifs were determined by polymerase chain reaction (PCR) and sequencing and VacA alleles by PCR. *cagPAI* functionality was assessed by interleukin (IL)-8 secretion, whereas CagA translocation was confirmed by Western blot detection of CagA, after antiphosphotyrosine immunoprecipitation of total protein lysates from *H. pylori*-infected AGS cells. Statistical analysis was pursued with multivariate logistic regression. Infection with CagA-positive strains carrying one EPIYA-C site was found to be an independent risk factor for gastroduodenal ulceration [odds ratio (OR): 4.647, 95% confidence interval (CI): 2.037–10.602], while the risk was 2-fold higher in mixed infections with isogenic strains harboring increasing EPIYA motifs. CagA species with

more EPIYA-C repeats exhibited higher tyrosine phosphorylation rates but did not contribute to elevated IL-8 secretion, or to increased neutrophilic or mononuclear infiltration in the antrum. Increasing EPIYA-C repeats in CagA were associated with highly vacuolating vacA isotypes (s1/i1/m1 or m2). *VacAs1* allele was related to increased activity of chronic antral gastritis (OR: 3.319, 95% CI: 1.449–7.600) and the *vacAi1* allele to greater chronic inflammatory infiltration (OR: 6.514, 95% CI: 2.298–18.878). In conclusion, CagA and VacA contribute to *H. pylori* infection in a coordinated manner, differentially affecting clinical phenotypes and the inflammatory response.

Abstract no.: P06.11
How *H. pylori* Deals with Nitrosative Stress. Searching for Novel Defense Systems

M. C. Justino,* I. G. Boneca† and L. M. Saraiva*

*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Oeiras, Portugal; †Institut Pasteur, Paris, France

The production of nitric oxide (NO) by the enzymatic activity of iNOS constitutes a weapon of the eukaryotic immune system to fight pathogens and plays a key role in host defense in most bacterial and parasitic infections. NO and derived reactive nitrogen species have a severe impact in the pathogen, causing protein and lipid nitrosylation, damage to iron centers, inactivation of transcription regulators, enzymes and ion channels, and DNA damage. However, successful pathogens are able to respond to such aggressions by eliciting several protective mechanisms that in the end allow them to elude the host response and cause disease.

The human pathogen *H. pylori* is known to elicit the immune response, through the activation of the iNOS enzyme, and is submitted to an additional source of NO that derives from the chemical decomposition of nitrite in the acidic stomach's environment. Nevertheless the mechanisms by which *H. pylori* can resist nitrosative stress remain poorly understood.

To further elucidate the *H. pylori* response to nitrosative stress we have started an exhaustive screening of strains deleted in genes of unknown function. For each strain, we analyzed the growth inhibition that is caused by NO in comparison to the parental strain *H. pylori* 26695. Our results show that at least three mutants have higher growth sensitivity to nitrosative stress, which make them good candidates for more thorough studies aiming to clarify their actual role in NO protection.

Abstract no.: P06.12
Mixed Infection with Different cagA-Positive *H. pylori* Strains in Iranian Gastrointestinal Patients

Z. Karimi, S. Saberi, M. Douraghi, Y. Talebkhan, A. Oghalaei and M. Mohammadi

Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

Introduction: Cytotoxin-associated gene product (CagA) displays a critical role in pathogenesis of *Helicobacter pylori* strains. Different

investigations show that 60% of Western and approximately all of the East Asian *H. pylori* strains are cagA positive. Therefore, only the presence of *H. pylori* cagA+ strains in various geographic regions with high prevalence of infection fails to be informative. Diversity in the 3' region of this gene leads to varying virulent strains associated with more severe diseases. In this study we investigated *H. pylori* cagA subtype status and the presence of multiple infections among Iranian gastrointestinal patients.

Methods: Totally, 166 *H. pylori*-infected patients including 29 gastric adenocarcinoma, 32 peptic ulcer disease, 91 nonulcer dyspepsia, and 14 normal were enrolled. cagA diversities were determined in 466 *H. pylori* isolates from at least two biopsy specimens from different locations of the stomach. Polymerase chain reaction amplification was performed using primers cag2F/cag4R. SPSS package was used for data analysis.

Results: Collectively, 50% of the examined patients suffered from multiple infections (harboring more than one cagA subtypes). Of these 166 patients, 466 isolates were recovered which produced 563 *H. pylori* strains of which 513 were cagA-positive in nine different categories with the following distribution: 400 bp (1.0%), 450 bp (5.3%), 500 bp (2.9%), 550 bp (58.3%), 600 bp (2.1%), 650 bp (26.5%), 750 bp (3.3%), 800 bp (0.2%), and 850 bp (0.4%).

Conclusion: This investigation points to the remarkable fact that half of Iranian dyspeptic patients are infected with more than one cagA subtype strain of *H. pylori* which cautions against the use of this genotyping technique in identifying high-risk patients.

Abstract no.: P06.13
Detection of CagA EPIYA Motifs in *H. pylori* DNA Extracted from Recently Collected, Frozen, or Deparaffinized Biopsies and Clinical Samples

C. Calà, T. M. Fasciana, C. Bonura, A. Vella, E. Di Carlo, S. Taormina and A. Giammanco

Dipartimento di Scienze per la Promozione della Salute G. D'Alessandro Sez. di Microbiologia, Università degli Studi di Palermo, Palermo, Italy

CagA is a major virulence factor of *Helicobacter pylori* that, once injected into the epithelial cells and phosphorylated on specific bacterial tyrosine residues within repeating EPIYA-A, -B, -C, and -D motifs, localizes to the plasma membrane and interacts with a number of intracellular effectors suggested to play an important role in *Helicobacter pylori* pathogenesis. EPIYA-D (in East Asian CagA) and EPIYA-C motifs (in Western CagA) are the main sites of CagA phosphorylation and the presence both of EPIYA-D or an increasing number of EPIYA-C motifs, rather than the general CagA positivity, has been associated with more severe gastroduodenal disease.

With the aim to analyze EPIYA motifs in 24 cagA+ *H. pylori* isolates and in a number of recently collected, frozen, or, in particular, deparaffinized biopsies and clinical samples, all obtained from 62 patients with different *H. pylori* pathology, we comparatively evaluated EPIYA profiles by polymerase chain reaction amplification using single sets of primers flanking the variable EPIYA coding region, or a single forward primer and multiple reverse primers specific for the individual EPIYA motifs. Only the primers originally employed by Rudi et al. (1988) to amplify the variable 3' region of the cagA gene allowed identification of EPIYA motifs in all biopsies and clinical samples. Multiple infections and EPIYA

profiles with more than one EPIYA-C motif, in some cases confirmed by DNA sequencing, were observed in 12 and 22 patients, respectively. As expected, the increasing numbers of EPIYA-C motifs were associated with more severe gastric pathologies.

Abstract no.: P06.14
Relationship Between *hrgA* Gene of *H. pylori* Infection in Gastritis and Peptic Ulcer Disease in Thailand

C. Kittittrakul,* R. Vilaichone,* S. Tumwasorn† and V. Mahachai‡

*Gastroenterology Unit, Department of Medicine, Thammasat University Hospital, Pathumthani, Thailand; †Department of Microbiology, Chulalongkorn University Hospital, Bangkok, Thailand; ‡Division of Gastroenterology, Department of Medicine, Chulalongkorn University Hospital, Bangkok, Thailand

Background and Aims: It has been identified that the putative *Helicobacter pylori* virulence factor, *hrgA* gene, might be a disease-specific marker for significant upper gastrointestinal tract diseases such as gastric cancer and peptic ulcer disease (PUD) in East Asian countries. Our aim was to study the relationship between *hrgA* gene of *H. pylori* infection and PUD in Thailand.

Methods: A total of 218 dyspeptic patients who underwent upper gastrointestinal endoscopy at Thammasat University Hospital, Thailand, during January 2007 to February 2008 were enrolled in this study. Two antral gastric biopsies were obtained for culture and *hrgA/hpyIIIR* status was determined by polymerase chain reaction using DNA expanded from a single colony.

Results: Forty-nine *H. pylori*-positive patients were enrolled in this study including 28 patients with gastritis and 21 patients with PUD. The mean age was 47.6 years (range 21–87 years). There were no significant differences in gender and age between patients with gastritis and PUD. *hrgA* gene was found in 39.3% in patient with gastritis and 42.9% in patients with PUD. However, the multivariate analysis did not identify statistical significant difference between these two groups (odds ratio = 1.2, 95% confidence interval: 0.4–3.6).

Conclusions: A direct relationship for *hrgA* gene and peptic ulcer disease could not be demonstrated in this study. Our data indicate that *hrgA* gene of *H. pylori* might not be associated with peptic ulcer disease in Thai patients.

Abstract no.: P06.15
Genetic Diversity in *dupA* Region of *H. pylori*

H. Matsuda,* Y. Ito,* T. Ohno,* S. Matsunaga,* M. Ohtani,* S. Lee,* T. Nemoto,* H. Suto,* Y. Yamazaki* and Y. Keida†

*University of Fukui, Eiheiji, Japan; †Okinawa Prefectural Central Hospital, Uruma, Japan

Aims: We examined whether there was a geographic diversity in the sequence of *dupA* (*jhp0917-jhp0918*), a 1839 bp gene in clinical isolates, and the upstream to *dupA*, by comparing various *dupA*-positive strains.

Methods: We chose eight Japanese *dupA*-positive strains isolated in two distant areas with different risks of gastric cancer (Okinawa and Fukui), four from patients in Okinawa [two with duodenal ulcer (DU), two with gastric cancer (GC)] and as many from Fukui (two with DU, two with GC). Polymerase chain reaction (PCR) primers were designed to amplify a fragment corresponding to nucleotides in G27 (a *dupA*-positive strain deposited in GenBank), including *jhp0917-0918* and those upstream. The PCR products were sequenced and compared with sequences of two other *dupA*-positive strains J99 and Shi470, deposited in GenBank.

Results: In Shi470, a strain isolated from Amerindian resident and related to East Asian strains, a continuous 2499 bp gene including *dupA* (*HP_04615*) were recognized. In Western strains, though G27 had a 2500 bp sequence homologous to *HP_04615*, it could not be a continuous gene by a stop codon. The upstream sequence to *jhp0917-0918* was completely different from those of the former two strains in J99, the other Western strain. In this study, all eight Japanese strains sequenced here possessed a continuous 2499 bp gene homologous to *HP_04615* (98.2–98.8%).

Conclusions: Our findings suggest that East Asian type of *H. pylori* strains have intact *dupA*. We could not find any significant difference in 2.5 kbp *dupA* region in strains from the two areas in Japan.

Abstract no.: P06.16
Influence of Chronobiologic Factors on Persistence of *H. pylori*

L. G. Bajenov

Republican Specialized Centre of Surgery, Tashkent, Uzbekistan

Sufficiently expressed seasonal prevalence of exacerbation of gastroduodenal diseases (GDD) causes expediency of the analysis of influence of chronobiologic factors on *Helicobacter pylori* detection frequency and the semination degree by them of the organism in patients with GDD. The results of bacteriologic investigation of 514 samples of gastric juice of patients with duodenal ulcer were analyzed on *H. pylori* presence. Mid-annual frequency of *H. pylori* revealing made 73.7%. However this parameter depending on the month of investigation changed in essential limits: from 29.3% in August up to 88.5% in February. The presence of four peaks of *H. pylori* isolation was determined: February, March, September, and November, whereas minimal levels of the isolation frequency of these microorganisms fell in April, August, October, and December. Peaks of gastric juice semination with *H. pylori* were observed in January, June, and October. The minimal values of this parameter were noted in January and October. It is possible to note highly expressed parallelism of the studied parameters; their significant reduction in August and then sharp rise in September ($p < .05$) attract special attention. The most expressed growth of these parameters fell in the beginning of autumn and spring seasons: the periods of GDD exacerbation, and reduction in parameters was noted during the periods of recession of frequency of these diseases and their relapses. The presented data serve as additional arguments in support of *H. pylori* etiologic value at GDD, as rise of *H. pylori* semination and their isolation frequency precede GDD exacerbation growth, and not the opposite.

Abstract no.: P06.17 Identification and Characterization of CagA EPIYA Motifs in Turkish Origin Strains

M. Guvenir,* O. Yilmaz,* A. Olivares,† C. Gonen,‡
M. Soyturk,‡ S. Sarioglu§ and G. I. Perez-Perez†

*Departments of Microbiology and Clinical Microbiology,
Medicine, Dokuz Eylül University, Izmir, Turkey; †New York
University School of Medicine, New York, New York, USA;

‡Department of Gastroenterology, Medicine, Dokuz Eylül
University, Izmir, Turkey; §Department of Pathology, Medicine,
Dokuz Eylül University, Izmir, Turkey

CagA is the most known virulence factors in *Helicobacter pylori* and increases the risk for disease. The aim of the study is to identify CagA EPIYA motifs in Turkish *H. pylori* strain and correlate with pathological findings in patients' biopsies.

Method: Endoscopies were performed in 62 adult patients. Antrum and corpus biopsies obtained for histology and culture. *H. pylori* strains were *vacA* genotyping and *cagA* status established as described (*Helicobacter*, 11, 2006). *H. pylori* *cagA*-positive polymerase chain reaction (PCR) products ranging from 370 to 570 bp were amplified (*J Clin Microbiol*, 45, 2007) and PCR products purified by QIAquick PCR kit (Qiagen) and sent to Macrogen (<http://www.macrogen.com>) for sequencing.

Results: We found 25 uninfected patients (41%) and 37 *H. pylori*-positive patients (59%). Uninfected patients had less chronic gastritis ($p = .015$) and were older than *H. pylori*-positive patients ($p = .005$). Among *H. pylori*-positive patients 17 (46%) were CagA positive. *H. pylori* CagA-positive patients had higher atrophy scores and intestinal metaplasia than *H. pylori* CagA-negative patients ($p = .02$). All *H. pylori* *cagA*-positive strains were *vacA* s1/m1 or s1/m2. In contrast, *H. pylori* *cagA*-negative strains were s2/m2 (75%). ABC EPIYA motif was common in Turkish strains (64.7%). However, no difference in pathology associated with number of EPIYA motifs was observed. Some patients (35.7%) were colonized with more than one strain (mix infection) based on phylogenetic analysis and variation in *vacA* and/or *cagA* genotype.

Conclusion: We confirmed the low prevalence of CagA-positive strains among Turkish patients and predominant ABC type in EPIYA motifs.

Abstract no.: P06.18 Associations of the Plasticity Region of *H. pylori* in Patients with Gastrointestinal Diseases

J. Yakoob, Z. Abbas, S. Naz, S. Abid and W. Jafri
Aga Khan, Karachi, Pakistan

The virulence genes of *H. pylori* outside *cag* pathogenicity island are described as plasticity region. The plasticity region genes include JHP0940, JHP0947, and JHP0986. The first two are associated with an increased risk of duodenal ulcer and gastric carcinoma (GC) while later with gastritis. We determined distribution of JHP0940, JHP0947, and JHP0986 and *cagA* in *H. pylori* isolates from patients with gastrointestinal diseases.

Methods: Of 43 isolates, 35 patients had gastritis, six peptic ulcers (PU) (four duodenal ulcers, two gastric ulcers), and two gastric carcinoma. DNA was extracted, and polymerase chain reaction was done for JHP0940, JHP0947, JHP 0986, and *cagA* gene using primers described before. Differences in proportion were assessed by Pearson chi-square, Fisher exact, or likelihood ratio test where appropriate. p value < .05 was significant.

Results: Of 43 patients, 29 (67%) were male, mean age 41 ± 13 years, range 22–66. JHP0940 was positive in 15 (35%), JHP 0947 in 14 (33%), JHP0986 in 10 (23%), and *cagA* in 21 (49%). JHP0986 was associated with *cagA* in four (40%) ($p = .52$), JHP0940 in seven (47%) ($p = .83$), and JHP0947 in nine (64%) ($p = .16$). JHP0947 was positive in five (83%) with PU, one (50%) with GC, and eight (30%) with gastritis ($p = .02$); JHP0940 was positive in two (100%) with GC, two (33%) with PU, and 11 (31%) with gastritis ($p = .11$) with GC and JHP0986 was associated with only gastritis in nine (26%) and with PU in one (17%) ($p = .51$).

Conclusion: JHP0947 gene was associated with peptic ulcer and gastric carcinoma. There was no association of *cagA* with hyperplasticity region genes; however, these are preliminary results and study is ongoing.

Abstract no.: P06.19 Detection of *H. pylori vacA* and *cagA* Virulence Genotypes by PCR

C. Özkul, Z. Sarıbaş and Y. Yılmaz
Hacettepe University Microbiology and Clinical Microbiology,
Ankara, Turkey

Prognosis of the *Helicobacter pylori* infections is strongly associated with the bacterial virulence factors. The most important virulence factors are proteins which are encoded by *cagA* and *vacA* gene. Generally, the presence of *cagA* and distribution of *vacA* allelic types have shown considerable geographic variation. The aim of this study was to determine the prevalence of *cagA* and *vacA* allelic types and to evaluate the association between clinical findings. The cetyltrimethyl-ammonium bromide (CTAB) method was used for the extraction of the DNA templates. In order to confirm the presence of *H. pylori* in stock strains, a 411-bp fragment of the *ureA* gene was amplified by polymerase chain reaction. After that, 120 isolates that are positive for *ureA* gene were amplified by primers which target specific sequences of *cagA* and *vacA* alleles (*s1a*, *s1b*, *s2*, *m1*, *m2*). The *cagA* were detected in 64 (53.3%) of the 120 strains. The *s1a*, *s1b*, and *s2* variants were detected in 70.1%, 2.8%, and 27.1%, respectively. Among middle(*m*) region variants, *m2* (65.5%) was found more prevalent than *m1* (33.6%). Both *m1* and *m2* genotypes were found together in one strain. The most frequently seen allelic combination was *s1a/m2* (35.6%) and *s1a/m1* (33.6%). Furthermore, no strain with the *s2/m1* combination was found. Although, there was no significant association between *cagA* positivity, *vacA* alleles, and clinical findings, an association between *vacA* allelic combinations and *cagA* positivity has been detected.