**Helicobacter pylori** Genotyping and Sequencing using Paraffin-Embedded Biopsies from Residents of Colombian Areas with Contrasting Gastric Cancer Risks

Liviu A. Sicinschi\(^{1*}\), Pelayo Correa\(^{1}\), Richard M. Peek, Jr. \(^{1}\), M. Constanza Camargo\(^{1}\), Alberto Delgado\(^{1}\), M. Blanca Piazuelo\(^{1}\), Judith Romero-Gallo\(^{1}\), Luis E. Bravo\(^{2}\), and Barbara G. Schneider\(^{1}\)

\(^{1}\)Division of Gastroenterology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee, USA; \(^{2}\)Department of Pathology, School of Medicine, Universidad del Valle, Cali, Colombia

**Background:** *cagA* positive and *vacA s1* and *m1* genotypes are associated with an elevated risk of gastric cancer (GC). We determined *H. pylori* genotypes using paraffin-embedded gastric biopsy specimens harvested from infected individuals and compared genotype distribution in two Colombian populations residing in geographic regions with a high and low GC incidence.

**Methods:** DNA from paraffin-embedded gastric biopsies from 107 adults was amplified using primers specific for *cagA*, for the *cag* “empty site”, for the *s* and *m* alleles of *vacA*, and for *H. pylori* 16S rRNA.

**Results:** *H. pylori* infection was detected by 16S rRNA assay in 97 (90.7%) biopsies. Complete genotyping of *cagA* and *vacA* was achieved in 87 (89.6%) cases. The presence of *cagA* was detected in 78 of 97 cases (80.4%); when considered separately, *cagA* and *vacA s* regions were not significantly associated with a particular geographic area. The *vacA m1* allele and *s1m1* genotypes were more common in the high GC risk area (P=0.037 and P=0.044 respectively), while the *vacA m2* allele and *s2m2* genotypes were more prevalent in the low risk area. The prevalence of *s1m1* genotypes and *cagA* positivity were 84.3% and 60.5% for high and low risk areas, respectively (P=0.011).

**Conclusions:** *H. pylori* *cagA* and *vacA* genotyping from paraffin-embedded gastric biopsies permitted reliable typability and discrimination. The more virulent *cagA* positive *s1m1* strains, as well as *vacA m1* genotype were more prevalent in high risk than in low risk areas, which may contribute to the difference in GC risk between those two regions.

**Key Words:** *H. pylori*, *cagA*, *s* and *m* *vacA* alleles, 16S rRNA, paraffin-embedded biopsies, PCR, gastric cancer, vacuolating cytotoxin, sequencing, synonymous and missense mutations.

* Corresponding author: Liviu A. Sicinschi, MD, PhD
  2215 Garland Ave.; Room 1005 MRB IV
  Nashville, TN 37232-0252; USA
  Phones: (615) 343-8626 (office)
  (504) 638-2912 (cell)
  Fax: (615) 343-6229
  E-mail: liviu.sicinschi@Vanderbilt.edu and/or
  lsicinschi@hotmail.com

**Running title:** *H. pylori* Genotyping from Gastric Biopsies