Infection, Genetics and Evolution 29 (2015) 68–74

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Analysis of the genotypic diversity of strains of *Helicobacter pylori* isolated from pediatric patients in Mexico

S. Mendoza-Elizalde [a,b], A.C. Cortés-Márquez [a,b], S. Giono-Cerezo [b], G. Zuñiga [c], A. Consuelo-Sánchez [d], P. Valencia-Mayoral [e], J.C. Vigueras-Galindo [a], G. Escalona-Venegas [a], J. Arellano-Galindo [a], N. Velázquez-Guadarrama [a,*]

[a] Laboratorio de Infectologia, Hospital Infantil de México Federico Gómez, México D.F., Mexico
[b] Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México D.F., Mexico
[c] Departamento de Zoología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, México D.F., Mexico
[d] Departamento de Gastroenterología y Nutrición, Hospital Infantil de México Federico Gómez, México D.F., Mexico
[e] Departamento de Patología, Hospital Infantil de México Federico Gómez, México D.F., México

ARTICLE INFO

Article history:
Received 7 August 2014
Received in revised form 29 October 2014
Accepted 1 November 2014
Available online 13 November 2014

Keywords:
Helicobacter pylori
cagA
babA2
vacA
EPIYA
Children

ABSTRACT

Genotypic differences in *Helicobacter pylori* play an important role in infection. We characterized the diversity of the *cagA*, *cagE*, *babA2*, and *vacA* genes in *H. pylori* strains isolated from pediatric patients and the relationship between these genes and clinical disease. Additionally, we employed the Neighbor-net algorithm to predict the behavior of the genotypes of the strains isolated from patients. Of 93 patients analyzed, 32 were positive for infection. A total of 160 *H. pylori* strains (five isolates per positive patient) were analyzed. A total of 91% and 83% of strains possessed the *cagA* and *cagE* genes, respectively. For the *vacA* gene, 84% of strains possessed the *s1* allele, 15% the *s2* allele, 81% the *m1* allele and 13.8% the *m2* allele. The *babA2* gene was present in 79% of strains. Infection with *H. pylori* strains with the *vacA* (*s1m1*) genotype was associated with risk of esophagitis and gastritis ($p = 0.0001$). The combination of *cagA* and *vacA* (*s1m1*) was significantly associated with abdominal pain ($p = 0.002$); however, EPIYA type was not significantly associated with abdominal pain. A total of 16 different genotypes were identified; the most common genotype was *vacAs1m1cagA+cagE+babA2+* (47.5%). A total of 84% of pediatric patients were infected by at least two and up to five different genotypes. The network recovered two genotype groups (A: strains with *vacAs1* and B: strains with *vacAs2*). The presence of multiple paths in the network suggests that reticulate events, such as recombination or reinfection, have contributed to the observed genotypic diversity.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Helicobacter pylori infects 35–70% of the global population (Perez-Perez et al., 2004) and plays an essential role in the development of gastric adenocarcinoma (Parsonnet et al., 1991). Infection is acquired during childhood, and prevalence gradually increases with age, reaching 50% in developing countries (Kivi and Tindberg, 2006; Torres et al., 2000). Transmission occurs primarily between mothers and their children and between siblings (Rocha et al., 2003). Person-to-person contact through the fecal/oral, oral/oral or gastric/oral pathways is the most commonly implicated mechanism, although water-borne transmission may be possible (Brown, 2000). The bacterium possesses virulence genes that contribute to the risk and severity of disease, of which the most important genotypic determinants are the *vacA*, *cagA*, and *babA2* genes. The *vacA* gene (vacuolating toxin VacA) is polymorphic and contains the signal sequence "*s*" (*s1* or *s2*), a middle region "*m*" (*m1* or *m2*) (Atherton et al., 1995), and the more recently identified intermediate "*i*" (*i1* or *i2*) region (Rhead et al., 2007). Cytotoxin-associated gene (*cagA*) is a marker for the cag Pathogenicity Island (*cag*-PAI), a toxin that enters host cells and disrupts normal cytoskeletal function (Akopyants et al., 1998; Censini et al., 1996; Hatakeyama, 2009; Kusters et al., 2006; Segal et al., 1999).

CagA is tyrosine-phosphorylate at multiple Glu-Pro-Ile-Tyr-Ala (EPIYA) sites present in the C-terminal region. Each of the EPIYA segments contains a single EPIYA site (EPIYA-A, -B, -C, or -D site), based on the sequence surrounding the EPIYA motif (Hatakeyama,

* Corresponding author at: Laboratorio de Infectología, Hospital Infantil de México Federico Gómez, Dr. Márquez 162, Col. Doctores, Cuauhtémoc, México D.F., 06720, Mexico. Tel.: +52 (55) 52289917x2081; fax: +52 (55) 55 88 45 49.
E-mail address: normave@himfg.edu.mx (N. Velázquez-Guadarrama).

http://dx.doi.org/10.1016/j.meegid.2014.11.002
1567-1348/© 2014 Elsevier B.V. All rights reserved.

2004). The BabA adhesin protein is encoded by the *babA* gene, which has two alleles (*babA1*, and *babA2*). However, *babA1* contains a 10-bp insertion, and thus only *babA2* is functionally active. The BabA2 adhesin protein participates in the adhesion of *H. pylori* to gastric epithelial cells and mucosal surfaces expressing the fucosylated blood group Lewis^b human antigen (Gerhard et al., 1999; Ilver et al., 1998).

H. pylori strains are classified as Type I, Type II and "Triple-positive". Type I strains express the *cagA*, and *vacAs1* genes, Type II strains lack the *cagA*, and *vacAs1* genes, and "Triple-positive" strains express the *vacAs1*, *cagA*, and *babA2* genes (Gerhard et al., 1999; Xiang et al., 1995).

Of those infected with *H. pylori*, an estimated 5–68% are infected with mixed genotypes (Secka et al., 2011) due to the high mutation rate of *H. pylori* and the frequent exchange of genetic material that occurs when a single patient is infected with multiple *H. pylori* strains (Falush et al., 2001; Kennemann et al., 2011; Kersulyte et al., 1999; Suerbaum et al., 1998). A range of *H. pylori* genotypes have been documented in children. Similar estimates of mixed-genotype infections in pediatric patients have not been performed in Mexico. However, the high seropositivity (80%) and frequency of recombination between strains (Falush et al., 2001; Kersulyte et al., 1999; Morelli et al., 2010) indicates that *H. pylori* is present at an early age.

The aim of this study was to characterize the diversity of virulence genes (*cagA*, *cagE*, *babA2*, and *vacA*) in *H. pylori* strains isolated from pediatric patients in Mexico and the relationship of these genes with clinical disease. Additionally, we employed the Neighbor-net algorithm to predict the behavior of the genotypes of strains in patients.

2. Materials and methods

2.1. Patients

To analyze the diversity of virulence genes of *H. pylori* strains isolated from pediatric patients, bacterial cultures were performed from 93 gastric biopsies obtained from patients at the Department of Gastroenterology and Nutrition of the Hospital Infantil de México Federico Gómez who were diagnosed with dyspepsia and gastroesophageal reflux disease by low panendoscopy. Among the patients, 47% were schoolchildren, 28% were preschool children, 22% were adolescents and 3% were infants. The minimum age was 10 months, the maximum age was 17 years and 7 months, and the median age was 8 years and 7 months. Clinical diagnoses of gastritis, duodenitis, and esophagitis were determined by endoscopic evaluation.

The committees of ethics, biosafety and scientific at the Health Institute approved the study protocol in advance. All parents were informed about the nature of the study and provided written consent.

2.2. H. pylori culture

Antrum gastric biopsy specimens from all patients were homogenized, inoculated onto Casman agar plates (BD BBL® MD, USA) supplemented with 5% horse blood and antibiotics (3 mg/mL vancomycin, 5 mg/mL trimethoprim and 2 mg/mL amphotericin B) and cultured under microaerophilic conditions (5% O_2, 5% CO_2, 85% N_2, and 10% partial humidity) at 37 °C for 5–7 days. Bacterial identification was based on colony morphology, Gram stain, urease, catalase and oxidase tests. Five colonies isolated from each patient were stored at −70 °C in 1.5 mL of Brucella broth (BD BBL) supplemented with 10% fetal bovine serum and 25% glycerol.

2.3. Detection of virulence genes by PCR

Genomic DNA was extracted from cultured *H. pylori* using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA,) following the manufacturer's instructions. DNA was quantified in an Epoch Microplate Spectrophotometer (BioTek, Vermont, USA), and DNA integrity was evaluated by electrophoresis in 1% agarose gels.

H. pylori was identified by the presence of the *glmM* gene as described by Smith et al. (2004). The *vacA* (*s1*, *s2*, *m1*, and *m2*), *cagA*, *cagE*, and *babA2* genes were amplified by PCR under the conditions described by Atherton et al. (1995), Kauser et al. (2004) and Mizushima et al. (2001). Amplification was performed in a reaction volume of 25 μL of Master Mix (Promega) containing 100 ng of bacterial DNA, 2.5 mM $MgCl_2$, 10 mM dNTPs, 2 U of Taq DNA polymerase, 20 pmol of each primer and nuclease-free water in a Thermo Hybaid thermal cycler (PCR Express, CA, USA). The PCR products were separated by electrophoresis in 1% agarose gels at 80 V, followed by staining with ethidium bromide and imaging under UV illumination (ChemiDoc transilluminator, BIO-RAD, USA). DNA from *H. pylori* ATCC strain 43504 was included as a positive control.

2.4. Amplification of the 3′ variable region of cagA

The 3′ variable region of the *cag*A gene was amplified by PCR. Briefly, 100 ng of DNA was added to 1% Taq DNA polymerase buffer solution (50 mM KCl, and 10 mM Tris–HCl, pH, 8.0), 1.5 mM $MgCl_2$, 100 μM each deoxynucleotide, 1.0 U of Platinum Taq DNA polymerase (Invitrogen, Life Technologies, Brazil), and 10 pmol of each primer in a total volume of 25 μL. The primers used were previously described by Rudi et al. (1998). The reaction conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 50 s and annealing and extension for 160 s at 72 °C, and a final extension at 72 °C for 2 min. The PCR products were separated by electrophoresis in a 1.5% (wt/vol) agarose gel.

2.5. Sequencing of the 3′ variable region of cagA

PCR products were purified using ExoSap IT® (Affymetrix, Cleveland, OH, USA) according to the manufacturer's recommendations. The purified products were sequenced using a BigDye Terminator v3.1 Cycle Sequencing kit in an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences obtained were aligned using the CAP3 Sequence Assembly Program (available from: http://pbil.univ-lyon1.fr/cap3.php). After alignment, the nucleotide sequences were translated into amino acid sequences using the Blastx program (available from: http://blast.ncbi.nlm.nih.gov/Blast.cgi) and compared with sequences deposited in GenBank (http://www.ncbi.nlm.nih.gov/Genbank/).

2.6. Statistical analysis

We compared clinical variables between genotypes using the χ^2 test. For the size of our study, we used Fisher's exact test. A p value of <0.05 was considered statistically significant.

2.7. Genetic relatedness of H. pylori genotypes

The positive or negative amplification of the main virulence genes was coded as binary data: presence (1) or absence (0). The combination of these gene amplifications was used to designate the integrated genotype of each strain. All identified genotypes were included in the genetic relatedness analysis. Because it is highly likely that *H. pylori* undergoes recombination events, a

Download English Version:

https://daneshyari.com/en/article/5909311

Download Persian Version:

https://daneshyari.com/article/5909311

Daneshyari.com