Helicobacter pylori Virulence Factor Genotypes in Children in the United States: Clues about Genotype and Outcome Relationships[▽]

Yoshio Yamaoka, Rita Reddy, and David Y. Graham*

Michael E. DeBakey Veterans Affairs Medical Center and Baylor College of Medicine, Houston, Texas 77030

Received 19 January 2010/Returned for modification 30 March 2010/Accepted 20 April 2010

The most common *Helicobacter pylori* genotype among 37 U.S. children was *cagA* positive, *vacA* s1m1, and *oipA* “on” ($n = 17$, 45.9%), followed by *cagA* negative, *vacA* s2m2, and *oipA* “off” ($n = 8$, 21.6%), similar to the pattern in adults. *cagA* positivity was more common in blacks than in whites (i.e., 100% versus 56.5%, $P = 0.032$).

Infection with *Helicobacter pylori* is etiologically associated with gastritis, peptic ulcer disease, gastric atrophy, and gastric cancer. *H. pylori* is thought to be typically acquired in childhood, with infection continuing for decades if not lifelong. This long bacterial host association involves countless generations of bacteria which are thought to continually evolve as the intragastric conditions change, such that those best suited for the local conditions outgrow and replace less suited neighbors. There has been considerable interest in the molecular epidemiology of *H. pylori*'s putative virulence factors, especially CagA, VacA, and OipA (outer inflammatory protein A) (8, 11, 12). However, there are few studies of children and only one previous study investigating the relationship between *H. pylori* virulence factors and ethnic groups of children in the United States (4–6, 11). This study reports the patterns of *H. pylori* virulence factor genotypes in children of different ethnic groups in the United States.

The biopsy specimens and cultures were obtained as part of a multicenter study from 5 widely dispersed sites in the United States. The study was designed to validate the [¹³C]urea breath test for the diagnosis of *H. pylori* infection in children aged between 2 years and 17 years and 11 months (2). Symptomatic children scheduled for endoscopy were enrolled. Gastric biopsy specimens were evaluated by histology, rapid urease testing, and culture of *H. pylori* using established techniques (2). *H. pylori* culture isolates were evaluated for *cagA* and *vacA* genotypes using established PCR assays as previously described (9). The number of EPIYA (Glu-Pro-Ile-Tyr-Ala) repeat motifs in the 3' region of the *cagA* gene was evaluated using PCR as previously described (7). OipA is a member of the large outer membrane protein family whose functional status is regulated by slipped-strand mispairing based on the number of CT dinucleotide repeats in the 5' region of the gene (a switch status of “on” indicates the gene is functional, and a switch status of “off” indicates it is nonfunctional) (10). The 5' region of the *oipA* gene was amplified using previously described primers

(10), and the PCR fragments were purified and directly sequenced at Macrogen, Ltd., in Seoul, South Korea.

Forty-eight of 176 children enrolled were *H. pylori* infected, based on two positive tests or a positive *H. pylori* culture. The mean age was 11.5 years (range, 3.2 to 17.9 years). Thirty-seven were *H. pylori* culture positive. None had atrophic gastritis. Only one patient had a significant endoscopic abnormality, a duodenal ulcer (*cagA* positive, *vacA* s1m2, and *oipA* on). The most common *H. pylori* genotype was *cagA* positive, *vacA* s1m1, and *oipA* on ($n = 17$, 45.9%), followed by *cagA* negative, *vacA* s2m2, and *oipA* off ($n = 8$, 21.6%), *cagA* positive, *vacA* s1m2, and *oipA* on ($n = 5$), *cagA* positive, *vacA* s1m1, and *oipA* on ($n = 3$), *cagA* negative, *vacA* s2m2, and *oipA* on ($n = 2$), *cagA* negative, *vacA* s1m1, and *oipA* on ($n = 1$), and *cagA* positive, *vacA* s1m2, and *oipA* off ($n = 1$) (Table 1). Overall, 70% of strains were *cagA* positive, which is similar to is the proportion found in U.S. adults (6).

The frequency of *cagA* positivity was significantly higher in blacks than in whites (i.e., 100% versus 56.5%, $P = 0.032$ by Fisher's exact test). Three prior studies have examined the relationship between *cagA* status and ethnic groups (black versus white) in U.S. adults (4, 6, 11). Two of the three studies reported that the prevalence of the *cagA* gene was significantly higher in blacks than in whites (4, 11), which is in agreement with the current study of children.

It is currently thought that the basic genotype acquired in childhood remains throughout life. This notion is supported by studies of migrants, who typically show the same pattern as those remaining in the home country, and studies of families, where siblings tend to have similar strains. This concept has also been the basis for the use of *H. pylori* genotyping to trace the migration of humans throughout the world (e.g., out of Africa) (3). However, as noted above, strains are expected to evolve based on the changing environment of the stomach (i.e., development of atrophic gastritis). The number of EPIYA repeat motifs in the 3' region of the *cagA* gene has been related to virulence (i.e., risk of gastric cancer) (7). It was proposed that this change occurred in response to atrophy rather than being responsible for atrophy, as the development of atrophy allowed the more acid-susceptible multi-EPIYA repeat-containing strains to survive and become dominant (7). A recent report in which the number of EPIYA repeat motifs was evaluated longitudinally in three families confirmed that this can

* Corresponding author. Mailing address: Michael E. DeBakey Veterans Affairs Medical Center, RM 3A-320 (111D), 2002 Holcombe Boulevard, Houston, TX 77030. Phone: (713) 795-0232. Fax: (713) 790-1040. E-mail: dgraham@bcm.tmc.edu.

[▽] Published ahead of print on 26 April 2010.

TABLE 1. Relationship between *H. pylori* genotype and ethnic group

Race	No. of children with indicated results for <i>cagA</i> , <i>vacA</i> , and <i>oipA</i> ^a							Total
	Pos, s1m1, on	Neg, s2m2, off	Pos, s1m2, on	Pos, s1m1, off	Neg, s2m2, on	Neg, s1m1, on	Pos, s1m2, off	
White	8	8	3	1	1	1	1	23
Black	8	0	1	2	0	0	0	11
Asian	1	0	1	0	1	0	0	3
Total	17	8	5	3	2	1	1	37

^a Pos, positive; Neg, negative.

occur (1). The results in children are also consistent with this notion, as all *cagA* genes studied contained three EPIYA motifs ($n = 26$), which differs from the results in the U.S. adult patients, where four or more EPIYA motifs were found in 21% of those studied (17/81) (7). Future studies comparing *cagA* gene structure between children and adults in the same population would be useful to address whether the strains thought to be more virulent in relation to the development of gastric cancer predated the development of atrophic gastritis or out-competed other bacteria as a consequence of the changes in the intragastric environment.

In summary, a study of the relationship between *H. pylori* virulence factor-associated genotypes in relation to ethnic groups of children in the United States found that the prevalence of the *cagA* gene was significantly higher in blacks than in whites. Conclusions drawn from characterization of strains from patients after the development of a clinical *H. pylori* outcome (e.g., gastric cancer) may be misleading, as they and the outcome may both reflect changes in the intragastric environment rather than a cause and effect relationship.

The study was supported in part by a grant from Otsuka Pharmaceutical Co., Tokyo, Japan. D. Y. Graham receives support from the Office of Research and Development, Medical Research Service Department of Veterans Affairs, and from Public Health Service grant DK56338 which funds the Texas Medical Center Digestive Diseases Center. D. Y. Graham and Y. Yamaoka are supported in part by grant DK62813 from the NIH.

D. Y. Graham is a paid consultant for Otsuka Pharmaceuticals and until October 2009 received royalties on the Baylor College of Medicine patent covering materials related to the [¹³C]urea breath test. He is also a consultant for Novartis in relation to vaccine development for treatment or prevention of *H. pylori* infection.

The contents of this paper are solely the responsibility of the authors and do not necessarily represent the official views of the VA or NIH.

We thank the members of the United States Pediatric Urea Breath Test trial who provided the biopsy specimens for culture.

REFERENCES

- Argent, R. H., R. J. Thomas, D. P. Letley, M. G. Rittig, K. R. Hardie, and J. C. Atherton. 2008. Functional association between the *Helicobacter pylori* virulence factors VacA and CagA. *J. Med. Microbiol.* **57**:145–150.
- Elitsur, Y., V. Tolia, M. A. Gilger, J. Reeves-Garcia, E. Schmidt-Sommerfeld, A. R. Opekun, H. El-Zimaity, D. Y. Graham, and K. Enmei. 2009. Urea breath test in children: the United States prospective, multicenter study. *Helicobacter* **14**:134–140.
- Linz, B., F. Balloux, Y. Moodley, A. Manica, H. Liu, P. Roumagnac, D. Falush, C. Stamer, F. Prugnolle, S. W. van der Merwe, Y. Yamaoka, D. Y. Graham, E. Perez-Trallero, T. Wadstrom, S. Suerbaum, and M. Achtman. 2007. An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* **445**:915–918.
- Parsonnet, J., M. Replogle, S. Yang, and R. Hiatt. 1997. Seroprevalence of CagA-positive strains among *Helicobacter pylori*-infected, healthy young adults. *J. Infect. Dis.* **175**:1240–1242.
- Talarico, S., B. D. Gold, J. Fero, D. T. Thompson, J. Guarner, S. Czinn, and N. R. Salama. 2009. Pediatric *Helicobacter pylori* isolates display distinct gene coding capacities and virulence gene marker profiles. *J. Clin. Microbiol.* **47**:1680–1688.
- Tham, K. T., R. M. Peek, Jr., J. C. Atherton, T. L. Cover, G. I. Perez-Perez, Y. Shyr, and M. J. Blaser. 2001. *Helicobacter pylori* genotypes, host factors, and gastric mucosal histopathology in peptic ulcer disease. *Hum. Pathol.* **32**:264–273.
- Yamaoka, Y., H. M. El-Zimaity, O. Gutierrez, N. Figura, J. G. Kim, T. Kodama, K. Kashima, D. Y. Graham, and J. Kim. 1999. Relationship between the *cagA* 3' repeat region of *Helicobacter pylori*, gastric histology, and susceptibility to low pH. *Gastroenterology* **117**:342–349.
- Yamaoka, Y., S. Kikuchi, H. M. El Zimaity, O. Gutierrez, M. S. Osato, and D. Y. Graham. 2002. Importance of *Helicobacter pylori oipA* in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. *Gastroenterology* **123**:414–424.
- Yamaoka, Y., T. Kodama, M. Kita, J. Imanishi, K. Kashima, and D. Y. Graham. 1999. Relation between clinical presentation, *Helicobacter pylori* density, interleukin 1beta and 8 production, and *cagA* status. *Gut* **45**:804–811.
- Yamaoka, Y., D. H. Kwon, and D. Y. Graham. 2000. A M(r) 34,000 proinflammatory outer membrane protein (oipA) of *Helicobacter pylori*. *Proc. Natl. Acad. Sci. U. S. A.* **97**:7533–7538.
- Yamaoka, Y., H. M. Malaty, M. S. Osato, and D. Y. Graham. 2000. Conservation of *Helicobacter pylori* genotypes in different ethnic groups in Houston, Texas. *J. Infect. Dis.* **181**:2083–2086.
- Yamaoka, Y., M. S. Osato, A. R. Sepulveda, O. Gutierrez, N. Figura, J. G. Kim, T. Kodama, K. Kashima, and D. Y. Graham. 2000. Molecular epidemiology of *Helicobacter pylori*: separation of *H. pylori* from East Asian and non-Asian countries. *Epidemiol. Infect.* **124**:91–96.