Genotypic, Clinical, and Demographic Characteristics of Children Infected with *Helicobacter pylori*

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*Helicobacter pylori* isolates vary between geographic regions. Certain *H. pylori* genotypes may be associated with disease outcome. Thirty-eight children underwent diagnostic upper endoscopy at four medical centers and were retrospectively analyzed to determine if *H. pylori* virulence genes were associated with endoscopic disease severity, histologic parameters, and host demographics. The *H. pylori* virulence genotype was analyzed by a reverse hybridization line probe assay and type-specific PCR. Endoscopic ulcers or erosions were found in 17 (45%) patients, with 13 (34%) of these patients having antral nodularity. Histological gastritis, of varying severity, was present in all children. Four patients harbored more than one *H. pylori* strain: one subject had both *cagA*− and *cagA*-negative strains, while three patients harbored either two different *cagA*-negative strains (two children) or two *cagA*+ strains (one child). There were 28 (74%) *cagA*+ isolates; 19 were associated with the *vacA* s1b genotype, 7 were associated with the *vacA* s1a genotype, 1 was associated with the *vacA* s1c genotype, and 1 was associated with the s2 genotype. Of 14 *cagA*-negative isolates, 6 were *vacA* s2 genotype, 4 were *vacA* s1b, 3 were *vacA* s1a, and 1 was *vacA* s1c. Nine of ten (90%) Hispanics had similar *H. pylori* strains (*vacA* s1b,m1), and all Asian-Canadian children were infected by strains with *vacA* s1c genotype. No correlation between *H. pylori* strain and endoscopic or histopathologic abnormalities was found. This study provides a baseline framework of North American children and their *H. pylori* strains, serving as a powerful epidemiological tool for prospective investigations to better understand the transmission and evolution of diverse disease outcomes.

Using DNA fingerprinting, restriction fragment length polymorphism, and multilocus enzyme analysis, *Helicobacter pylori* strains isolated from adults have demonstrated considerable heterogeneity in selected genes (1, 3, 17, 34). The different *H. pylori* genes have shown distinct geographic distribution and correlation with severity of disease. van Doorn et al. (35, 38) demonstrated that *vacA* alleles have specific distributions among different ethnic groups and geographic regions; for example, the *vacA* s1c *H. pylori* strains are found exclusively in persons of Asian descent. Also, specific *H. pylori* genotypes (in particular, *cagA*, *vacA*, *cagE-picB*, and *iceA*) are considered more virulent strains since they are associated with more severe gastroduodenal disease in adults (18, 25, 27, 37, 38, 41, 43). For example, *vacA* type s1a strains have been isolated more frequently in adults with peptic ulcer disease and are associated with increased gastric epithelial damage (2, 41). An additional *H. pylori* virulence gene, *iceA* (induced by contact with epithelium), has been more commonly observed in *H. pylori* strains isolated from adults with peptic ulcer disease compared to those with gastritis alone (6, 7, 38).

The *cagA* gene is closely associated with the *vacA* s1 genotype and is considered a marker for severe host disease (6, 7, 21, 24, 26, 28, 30, 38). Using serology, Eltsur et al. (13) estimated that the prevalences of anti-CagA antibodies among asymptomatic children were 54 and 69% among symptomatic children (*P < 0.05*). More recently, Yahav et al. (42) has shown that anti-CagA seropositive *H. pylori*-infected children have more severe gastroduodenal disease and worse outcomes (*i.e.*, more difficult to eradicate and longer time for disease resolution) than *H. pylori*-infected children who are CagA seronegative.

Studies of genetic variability of *H. pylori* in children have been restricted to single-center, serological analysis of the *cagA* pathogenicity island (8, 9, 22, 26). Moreover, there have been no studies that have evaluated pediatric *H. pylori* isolates in correlation with quantitative histopathologic data in infected children. Accordingly, a multicenter pediatric study was undertaken to determine if different *H. pylori* genotypes are associated with disease severity, are seen in specific ethnic groups, or have a restricted geographic distribution. In this retrospective study, we investigated the role of the virulence genes *cagA*, *vacA*, and *iceA* in gastroduodenal disease in children by ana-
lyzing *H. pylori* strains obtained from four different sites in North America using PCR and a reverse hybridization assay. The *H. pylori* genotype was also correlated with demographic, endoscopic, and histologic data.

**MATERIALS AND METHODS**

**Patient population, endoscopy, and pathology data.** This study included a random sample of patients from four centers (Miami Children's Hospital, Miami, Fla.; Rainbow Babies and Children's Hospital, Cleveland, Ohio; Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; and Children's Healthcare of Atlanta at Egleston, Atlanta, Ga.) in which an *H. pylori* culture was available for genotyping. The *H. pylori* specimen was obtained during a diagnostic fiberoptic upper endoscopy, which was performed at the discretion of the pediatric gastroenterologist because of the subject's persistent gastrointestinal symptoms and signs. The study cohort was accrued over a 3-year study period, and patients were selected for analysis using a random numbering scheme. All patients were treated at each center with eradication *H. pylori* therapy as described previously (11).

Endoscopic diagnoses in the stomach were defined, for the purpose of this study, as follows: normal gross appearance, erosions (anatomical location in stomach was designated), ulcers (anatomical location), and nodularity (anatomical location). Endoscopic pictures from each endoscopy were independently reviewed by one of the authors (B.D.G.) in order to standardize endoscopic reporting for each patient. From each child, a minimum of four endoscopic gastric biopsies were obtained: one for on-site rapid urease testing, one for culture, and at least two for histopathology (one from the antrum and another from the corpus).

The two biopsies used for histopathologic evaluation were formalin fixed, paraffin embedded, and stained with hematoxylin and eosin. In order to obtain uniform grading of the inflammatory infiltrate, one pathologist retrospectively reviewed the biopsies using the visual analog scale from the Updated Sydney Classification of Gastritis (10). The Updated Sydney Classification of Gastritis grades the following histopathological features on a scale that goes from absent to marked, namely, (i) the amounts of bacteria, (ii) neutrophils, and (iii) mononuclear inflammatory cells, as well as (iv) the degree of atrophy and intestinal metaplasia present in a biopsy. When necessary, silver impregnation stains were used to confirm identification of bacteria in the gastric biopsies. For the final pathology diagnosis, the gastritis was classified as either chronic active, if both neutrophils and mononuclear inflammatory cells were present, or chronic if only mononuclear cells were seen.

**Bacterial cultures.** Three centers (Cleveland, Toronto, and Atlanta) had the microbiological laboratory capability to permit on-site primary cultures. In these centers, biopsies (ca. 0.1 to 0.2 mg/biopsy) were homogenized under aseptic conditions in either 1.5 ml of sterile saline or transport medium (vial containing 1.5 ml of brucella broth and 20% sterile glycerol) and stored at 4°C for up to 24 h before processing. For DNA extraction, bacteria were resuspended by suspension in 2 ml of sterile 0.9% NaCl solution and sedimented by centrifugation at 10,000 rpm for 2 min. For DNA extraction, bacteria were resuspended by suspension in 2 ml of sterile 0.9% NaCl solution and sedimented by centrifugation at 10,000 rpm for 2 min. For DNA extraction, bacteria were resuspended by suspension in 2 ml of sterile 0.9% NaCl solution and sedimented by centrifugation at 10,000 rpm for 2 min. 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cases showed two *H. pylori* strains in the same isolate since there was evidence of different *vacA* s and *m* genes. Of the 34 cases where only one *H. pylori* strain was identified, 25 (74%) were *cagA*1 and 9 (26%) were *cagA* negative. The *vacA* and *iceA* genotypes varied through the *H. pylori* strains. Of the 28 *cagA*1 isolates, 19 (68%) were associated with the *vacA* s1b genotype, 7 (25%) were associated with the *vacA* s1a genotype, 1 (4%) was associated with the *vacA* s1c genotype, and 1 (4%) was associated with the s2 genotype. Among the 14 *cagA*-negative isolates, 6 (43%) were *vacA* s2 4 (29%) were *vacA* s1b, 3 (21%) were *vacA* s1a, and 1 (7%) was *vacA* s1c. The *vacA* s2 genotype was always associated with the *vacA* m2 genotype.

**Correlation of *H. pylori* genotype and demographic data.** Two of the Caucasian children had more than one *H. pylori* isolate; both had two *cagA*-negative strains. The majority of Caucasian children (10 of 16) were *cagA*1 (5 from Cleveland, 2 from Atlanta, and 3 from Toronto). Their *vacA* and *iceA* genotypes were varied. Six Cuban-American children from Miami had *cagA*+ strains and *vacA* s1b, m1 alleles. Two of the black children had more than one *H. pylori* strain; one was from Cleveland, and the other was from Atlanta. Seven of the isolates from six of the black children were *cagA*+ and two were *cagA*-negative strains. The three children from Georgia of mixed black-white race showed a variety of *cagA*, *vacA*, and *iceA* genotypes. The two children from Asian descent were from Toronto and showed different *cagA*, *vacA* m, and *iceA* genotypes; however, they shared the same *vacA* s1c gene.

**Correlation of *H. pylori* genotype with endoscopic and histopathologic diagnoses.** Endoscopy demonstrated ulcers or erosions in 12 (32%) of the children with *cagA*+ *H. pylori* and in 5 (13%) of the children with *cagA*-negative strains (*P* > 0.05; not significant). Gastric nodularity was present in 9 (24%) of the children with *cagA*-negative strains (*P* > 0.05; not significant). Endoscopies classified as having normal gastric mucosa were seen in five (13%) children with *cagA*+ isolates and in three (8%) children with *cagA*-negative *H. pylori* strains (*P* > 0.05; not significant). Among the 33 patients for whom pathology was available, marked gastritis was present in 13 (39%) children with *cagA*+ *H. pylori* and in 9 (27%) children with *cagA*-negative strains (Fig. 2). Moderate gastritis was seen only in 4 (12%) of children with *cagA*+ *H. pylori*. Mild inflammation was demonstrated in six (18%) children with *cagA*+ *H. pylori* and in two (6%) children with *cagA*-negative strains. As mentioned previously, atrophy was seen in two patients of Hispanic decent, both of whom harbored *cagA*+ isolates. Atrophy was also demonstrated in one of the children of Asian decent; this patient had a *cagA*-negative isolate.
DISCUSSION

This study is the first multicenter genotypic analysis of *H. pylori* strains obtained from pediatric populations. We found a variety of *H. pylori* genotypes but could not demonstrate an association between the strain genotype and either the endoscopic features or the histopathologic findings of infected children. This lack of correlation between the *H. pylori* genotype and the pediatric gastroduodenal disease may be due, in part, to a highly selected symptomatic population evaluated upon referral to the pediatric gastroenterologists at tertiary-care, academic centers. Conversely, the bias of our patient population should have exaggerated the potential relationships between genotype and virulence. Specifically, since we only studied individuals who presumably had highly virulent disease resulting in clinical symptoms which then resulted in the child’s referral to the subspecialist and endoscopic evaluation, the fact that we saw no relationship between genotype and *H. pylori* virulence genes is even more significant.

In our study cohort, only the ethnic or racial origin of the infected host seemed to be a factor, which correlated with the *H. pylori* strain genotype. All children resided in North America and, despite the relative small sample size, the diversity of ethnic backgrounds is relatively reflective of the demographics found in both the United States and Canada, adding validity to the correlation between ethnicity or race and the *H. pylori* strain genotype. This retrospective study facilitated the creation of a network of medical centers with specific capabilities to determine a baseline of patients and *H. pylori* genotypes that will enable us to plan a prospective multicenter study. Prospective investigations employing this multicenter cohort will yield the numbers needed to ascertain the overall impact of *H. pylori* genotype on the spectrum of pediatric gastroduodenal disease. Moreover, the inclusion of additional centers, prospective enrollment, and better representation of the diverse ethnic makeup of North America and, thereby, the ability to sample multiple generations of children from different ethnic backgrounds may provide additional insight into the evolution of *H. pylori* genotypes in different populations worldwide.

Recently, investigators have demonstrated that distinct *H. pylori* genotypes have specific geographic distributions (35, 37, 38). In Europe, for example, a distribution gradient of the vacA s1 subtypes has been observed (i.e., vacA s1a genotype in individuals from northern Europe, England, Ireland, and Scotland), whereas in Central and South America, virtually all *H. pylori* strains contained the vacA s1b genotype and in East Asia the subtype s1c is observed most frequently (35, 38). In the present study, the two Asian children had a vacA s1c allele, and most of the *H. pylori* isolates from Hispanic children had a vacA s1b,m1 genotype. Although our study cohort was relatively small in size, these genotypes follow a geographic and ethnic distribution pattern similar to the one seen in adult populations.

This study highlights a number of important observations, such as providing evidence that multiple *H. pylori* genotypes can occur in infected children. It is possible that children, when first acquiring the infection, are colonized by multiple *H. pylori* strains. It has been postulated that over time, through natural selection influenced by host and bacterial factors, one specific *H. pylori* genotype predominates in infected adults (4, 20, 23, 31). This may be the reason why, in our previous studies, *H. pylori*-infected children have been found to have greater numbers of organisms compared to infected adults (40). Additionally, data from a nonhuman primate model of *Helicobacter* infection provide support for this hypothesis. Rhesus monkeys challenged with a mixture of seven genetically distinct *H. pylori* strains resulted in variable susceptibility to different genotypes during the acute phase of the infection compared to latter stages when one *H. pylori* strain predominated (12).

Onset of *H. pylori* infection is during childhood in most human populations (29). Although it is believed that both host factors and bacterial factors dictate eventual disease outcome, the natural history of *H. pylori* infection after childhood acquisition remains poorly characterized (5, 19, 20). In this study, only symptomatic pediatric patients were endoscoped, i.e., children with persistent upper gastrointestinal signs and symptoms warranting diagnostic upper endoscopy. Endoscopic abnormalities were evident in more than half of the children with *H. pylori* isolates which were cagA+, yet there were no significant differences in endoscopic or histologic diagnoses in those children harboring cagA compared to cagA-deficient strains. In this cohort of children, the prevalence of peptic ulcer disease was high for this age group. This is likely due to the retrospective selection of cases for study from tertiary care referral centers (i.e., selection bias). Future studies that incorporate a greater number of pediatric gastrointestinal centers from different geographic regions are necessary to eliminate some of this bias to the study sample and thus to better evaluate childhood gastroduodenal disease in correlation with bacterial genotype.

Finally, the observation of *H. pylori*-infected pediatric patients with atrophy and intestinal metaplasia is exceedingly uncommon. Pathologic diagnosis of atrophy has been controversial in adult populations because of the lack of strict diagnostic criteria, difficulties in performing the diagnosis in one biopsy, and poor reproducibility when assessing severity (14, 16, 32, 33). Clearly, prospective studies with larger numbers of children from multiple centers and geographic regions are needed to better define the spectrum of illness and natural history of disease following pediatric *H. pylori* infection, as well as to better understand the epidemiology and pathobiology of this infection; such studies are essential for developing more effective methods of eradication and prevention.