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NOTES

Identification of Enterohepatic *Helicobacter* Species in Patients Suffering from Inflammatory Bowel Disease

Ulrich R. M. Bohr,* Bernhard Glasbrenner, Anett Primus, Alexandra Zagoura, Thomas Wex, and Peter Malfertheiner

Department of Gastroenterology, Hepatology and Infectious Diseases, Otto von Guericke University, Magdeburg, Germany

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Using a group-specific PCR assay, we investigated the presence of enterohepatic *Helicobacter* species in gut specimens from patients with inflammatory bowel disease. Enterohepatic *Helicobacter* species were detected in 12% (3 of 25) of the patients with Crohn's disease, in 17% (3 of 18) of the ulcerative colitis samples, and in 4% (1 of 23) of the controls.

Members of the family *Helicobacteraceae* are able to colonize various ecological niches in the gastrointestinal tract (19). With respect to their preferential site of colonization, *Helicobacter* species are divided into two subgroups. The better-known gastric *Helicobacter* species, which preferably colonize the host's stomach, represent only one-third of the known species of *Helicobacteraceae*. The remaining two-thirds of *Helicobacter* species are referred to as enterohepatic because they predominantly colonize the intestine and the hepatobiliary system (19). Recently, enterohepatic *Helicobacter* species have been discovered in inflammatory bowel disease (IBD) of rodents (9, 14, 16), carnivores (7, 8), and primates (10, 18). Infection experiments, moreover, demonstrated that enterohepatic *Helicobacter* species can trigger IBD in susceptible animals (4, 5, 13, 15). In order to investigate if enterohepatic *Helicobacter* species are also present in human IBD, we performed this prospective evaluation.

A series of 115 consecutive patients who underwent colonoscopy were screened for eligibility to participate in this study. Within this series, all patients with clinically established diagnosis of Crohn's disease ($n = 25$) or ulcerative colitis ($n = 18$) as well as a control group of patients defined by the absence of macroscopic or microscopic abnormalities ($n = 23$) were selected. The 49 remaining patients had other pathologies such as neoplasia, diverticulosis, or human immunodeficiency virus-associated enteritis and were excluded from further analysis. Of the three patient groups examined, the patients with Crohn's disease were significantly younger (average age, 41.0 ± 11.7 years) than the ulcerative colitis patients (average age, 51.7 ± 10.3 years). The patients without morphological changes were significantly older (average age, 65.1 ± 9.1 years) than both IBD groups.

* Corresponding author. Mailing address: Department of Gastroenterology, Hepatology and Infectious Diseases, Otto von Guericke University Magdeburg, Leipziger Strasse 44, D-39120 Magdeburg, Germany. Phone: 49-391-67-13100. Fax: 49-391-67-13105. E-mail: ulrich.bohr@medizin.uni-magdeburg.de.

Biopsy specimens from the terminal ileum and the colon of each patient were analyzed by a group-specific PCR assay with primers C97 (5′-GCTATGACGGGTATCC-3′) and H2 (5′-TCGCAATGAGTATTCCTCTT-3′) as previously described (2, 3). In order to identify the *Helicobacter* species, amplification products were sequenced as described before (3).

Nine of 25 (36%) of the Crohn's disease patients and 7 of 18 (39%) of the ulcerative colitis samples were positive for *Helicobacteraceae*, compared to 15 of 23 (65%) in the control group. DNA sequencing of the complete amplification product in these 31 positive samples revealed single *Helicobacter* species in 28 patients; two patients in the Crohn's disease group and one patient in the ulcerative colitis group were coinfected with two different *Helicobacter* species. All individual 16S rRNA gene sequences could be assigned to a known *Helicobacter* species, as documented in Fig. 1. *H. pylori* was identified in 27 patients, *H. fennelliae* in four patients, and *H. pullorum* in three patients. In two patients in the Crohn's disease group, *H. pullorum* was detected. An additional Crohn's disease patient in whom *Yersinia enterocolitica* was also detected in stool cultures was positive for *H. fennelliae*. In three patients with ulcerative colitis, *H. fennelliae* was identified. One patient in the control group was *H. pullorum* positive.

Altogether, enterohepatic *Helicobacter* species were detected in 12% (3 of 25) of Crohn's disease cases, in 17% (3 of 18) of ulcerative colitis cases, and in 4% (1 of 23) of the controls. In 32% (8 of 25) of the patients with Crohn's disease, in 28% (5 of 18) of the ulcerative colitis patients, and in 61% (14 of 23) of the controls, *H. pylori* DNA was detected in the gut mucosa. Figure 2 shows the proportion of patients with enterohepatic *Helicobacter* species and *H. pylori* in the individual patient groups. *H. pylori* was significantly more frequent in controls than in patients with IBD ($P = 0.02$). In contrast, enterohepatic *Helicobacter* species were more frequent in IBD, but this difference was not statistically significant.

The prevalence of enterohepatic *Helicobacter* species of 12 and 17% in Crohn's disease and ulcerative colitis patients, respectively, indicates that a significant portion of patients with

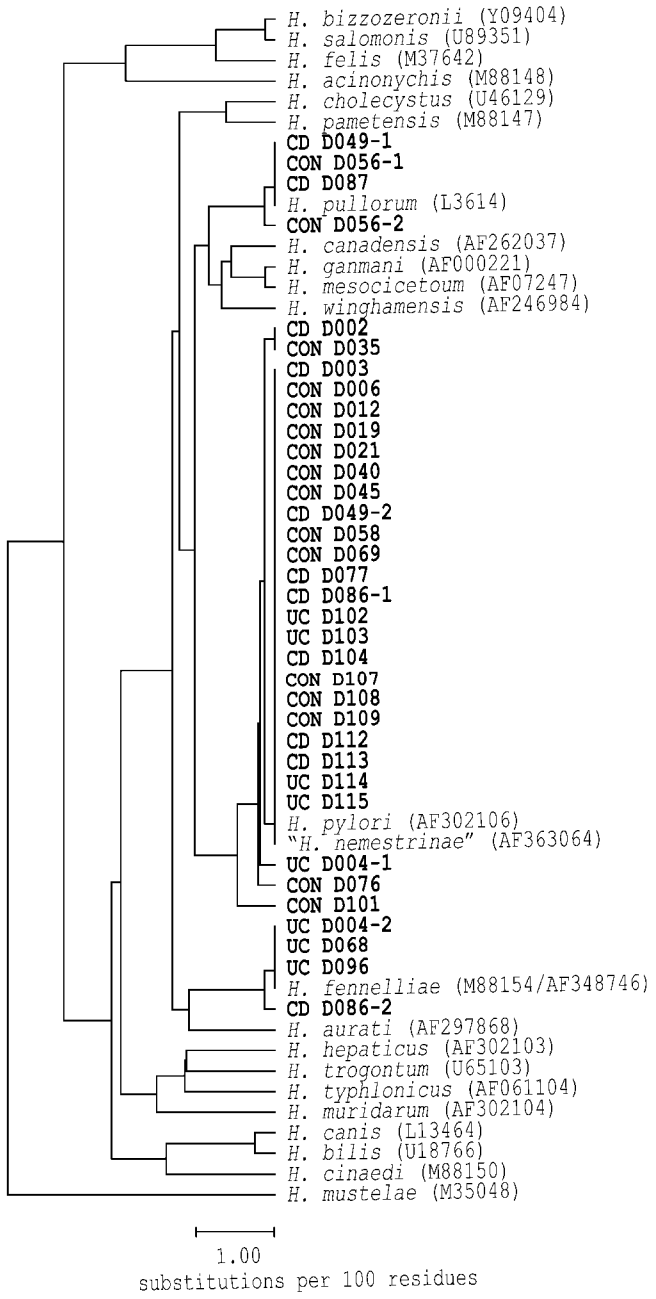

FIG. 1. Phylogenetic tree based on the evolutionary distance of the detected 16S rRNA sequences and *Helicobacter* reference strains. The scale bar is equal to 1 substitution per 100 residues, as determined by measuring the lengths of the horizontal lines connecting two species. Subjects are represented by bold letters that represent the diagnosis of Crohn's disease (CD), ulcerative colitis (UC), or control status (CON) and the patient code. A number after a dash indicates that several sequences were obtained from a single patient. The code in brackets after the names of the reference strains indicates the GenBank accession number of the corresponding 16S rRNA sequence.

IBD carry these potentially harmful bacteria. Interestingly, in patients with ulcerative colitis, only *H. fennelliae* was identified. Recently, *H. fennelliae* was also detected in homosexual men with proctitis and proctocolitis (21). These patients typically present symptoms that are similar to those in ulcerative colitis:

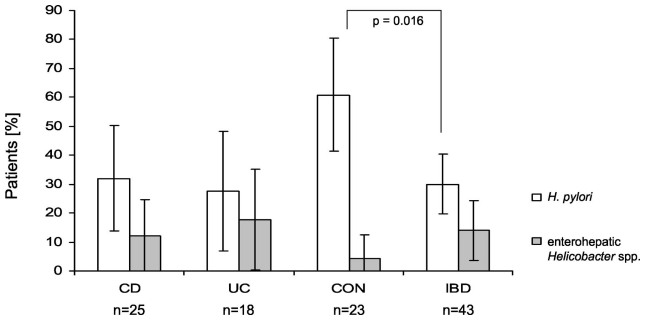

FIG. 2. Prevalence of *H. pylori* and enterohepatic *Helicobacter* species in Crohn's disease (CD) patients, ulcerative colitis (UC) patients, controls (CON), and both types of IBD patients combined (IBD). The error bars indicate the 95% confidence interval.

rectal pain, tenesmus, and diarrhea. As in ulcerative colitis, the symptoms are limited to the large bowel. In patients with Crohn's disease, *H. pullorum* was the dominant enteric *Helicobacter* species. Recently, *H. pullorum* was isolated from patients with gastroenteritis, in whom, as in Crohn's disease, the complete gastrointestinal tract is affected (20).

Another interesting aspect is the production of cytolethal distensing toxin by *H. pullorum* but not *H. fennelliae*. Cytolethal distensing toxin is an important pathogenicity factor that is also produced by *Campylobacter* species, pathogenic *Escherichia coli* strains, and *Shigella* spp. It causes cellular distension, cytoskeletal abnormalities, G_2/M cell cycle arrest, and cytolethality (23, 24). The expression of cytolethal distensing toxin by enterohepatic *Helicobacter* species could potentially affect the development of IBD. One could speculate that the production of cytolethal distensing toxin by *H. pullorum* in patients with Crohn's disease may play a role in the involvement of deeper layers of the bowel which is typical of Crohn's disease. In consequence, it seems logical that *H. fennelliae*, which lacks this toxin, is linked to ulcerative colitis, in which inflammation is usually limited to the mucosa.

During the review of our manuscript, another study that investigated the presence of *Helicobacter* species in human IBD in the United Kingdom was published (1). In that study, neither *H. pylori* nor other *Helicobacter* species could be detected in 30 patients with IBD. In an area where the prevalence of *H. pylori* is approximately 40 to 50%, one would have expected several *H. pylori*-positive patients. Therefore, the inability to detect *H. pylori* in that study is surprising and suggests a methodological problem or another bias. Our finding that the frequency of *H. pylori* is significantly lower in patients with IBD compared to controls is consistent with other studies that reported similar results with different methods (6, 11, 12, 17, 22). Since it has been reported that the low prevalence of *H. pylori* in patients with IBD is not due to therapeutic effects, infection with *H. pylori* might directly reduce the relative risk of IBD (17, 22). Possible mechanisms could be immunomodulatory effects, direct interactions with the intestinal mucosa, or mechanisms that prevent the colonization of the host by enterohepatic *Helicobacter* species.

We emphasize that this study was designed as a pilot study and was not intended to prove a causative pathogenic role of enterohepatic *Helicobacter* species in human IBD. Based on

our results, we conclude that these bacteria exist in at least a subgroup of patients with IBD. This is an important novel finding because intestinal bacteria are known to play an important role in human IBD and enterohepatic *Helicobacter* species are able to induce IBD in susceptible animals. It will now be important to investigate whether enteric *Helicobacter* species play a causative role in human IBD.

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