14 samples from noninoculated guinea pig brains as negative controls. All samples were assayed in a blinded fashion.

Detection of the *Borrelia* and IC amplification products was performed using the DELFIA (dissociation-enhanced lanthamide fluoroimmunoassay) time-resolved fluorescence hybridization assay (Perkin-Elmer Wallac, Gaithersburg, MD), as described elsewhere [7]. Probe sequences were as follows: *Borrelia*: 5′-GATGCACACCTTGGTGTTAATCAAAAG-3′; IC: 5′-GC-GATGCTGTCCGAAAACG-3′.

To assess the sensitivity and reliability of our PCR assay, the *Borrelia* PCR target region was cloned into the pCR 2.1 vector. A dilution series was performed, with the addition of 1750 ng human genomic DNA per reaction, and the target region was then amplified, using our PCR assay. Time-resolved fluorescence was measured to determine positive amplification reactions. The quantification of β-actin genes has been used to calculate that each cell in the trigeminal ganglia contains 15.6 pg of DNA [8]. Therefore, we calculated that our PCR sensitivity was at least 2 *Borrelia* genomes per 112,000 cells.

When our PCR method was used, no patients with AD or controls were positive for *Borrelia* species in the brain. Therefore, no brain sample in either group was positive by PCR analysis for *Borrelia* organisms, with a 95% confidence interval of 0%–20%. All 18 samples from guinea pig brains inoculated with *B. burgdorferi* were positive, whereas all 14 samples from noninoculated guinea pig brains were negative.

In summary, using a very sensitive PCR assay that is able to amplify a *Borrelia*-specific DNA target sequence from all *B. burgdorferi sensu lato* species known to cause disease in humans, we found no evidence of *Borrelia* organisms in brains of patients with AD.

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*Helicobacter pylori* Infection, *cagA* Status, and Duodenal Ulcer Disease in Children

To the Editor—I would like to comment on a recently published report by Queiroz et al. [1]. In this article, the authors described the significant association between *cagA*-positive status and duodenal ulcer disease in symptomatic children with *Helicobacter pylori* infection, and they also found a direct correlation between degree of inflammation and *cagA*-positive status. Interestingly, a close association between increasing age and *cagA* status, in *H. pylori*-positive children without duodenal ulceration, was reported. Unfortunately, a similar comparison in the duodenal ulcer–positive children could not be done, because all of them were *cagA* positive. Although the results of this report are consistent with those of studies in adults [2–5], the picture in the pediatric population is still unclear. On the basis of serology test results (*Helicoblot 2.0; GeneLabs Diagnostics, South America*), we reported that >50% of symptomatic and/or asymptomatic *H. pylori*-infected children were found to be positive for *cagA* antibody, but duodenal ulceration was found in only 9% (2 children, only one of whom was positive for *cagA* antibody) [6]. In addition, only a weak association was found between inflammatory index (assessed by the revised Sydney criteria) [7] and the presence of *cagA* antibody in symptomatic children. No association was observed between *cagA* status and age, sex, community location, or *H. pylori* density. Although the revised Sydney criteria [7] were used by Queiroz et al., intestinal metaplasia and *H. pylori* density parameters were not scored.

The association between *cagA* status and the development of duodenal ulcer disease (or between *cagA* status and the inflammatory index) in children with *H. pylori* infection is probably more complicated than we contemplate. From the epidemiological point of view, it is interesting to note that, in countries where *H. pylori* infection and *cagA*-positive status are very high, the rates of duodenal ulcer disease and gastric cancer are considerably low [8, 9]. These data suggest that other environmental factors are more crucial than *cagA* status for ulcer development. *H. pylori* infection seems to have a close association with increasing age. Older children with *H. pylori* in-
fection tended to harbor a more virulent bacterial strain [1]; may have a stronger immune response, as reflected by histology and antibody level [10, 11]; or may have pronounced signs, such as gastric nodularity, as determined by endoscopy [12]. Do microbiological mucosal factors, such as incubation time or host defense factors (favoring one strain of bacteria with time), have anything to do with increasing age? Did other factors, such as sex or compromised immune status, have a contributing effect on Queiroz et al.’s results? In their results, the authors noted that a significantly higher rate of duodenal ulcer was seen in boys than in girls (63% vs. 37%), but no association between sex and cagA status was found among ulcer-free children. Are sex hormones a factor in the H. pylori–duodenal ulcer story? Moreover, in their report, Queiroz et al. did not specify the nutritional status of the children, except to describe them as a cohort of children from a low socioeconomic status, with no history of underlying disorders. Has the environmental and/or nutritional status of the children contributed to the study results? Are there any other confounding factors—such as alcohol or tobacco use—to explain the higher rate of duodenal ulcer development and the uniformly cagA-positive strain found in those older children? For a complete investigation of the cagA status–duodenal ulcer association, all possible confounding factors, that is, sex, age, inflammatory index, and environmental/nutritional status, should be examined statistically, by multivariate regression analysis.

In summary, the report by Queiroz et al. is another attempt to examine the association between H. pylori strain and mucosal pathology (inflammation and/or duodenal ulceration) in children. I believe that the nature of this association is still open for speculation and debate, before the true correlation will be found. The statistical discrepancy between the very high rate (>50%) of cagA-positive status [6, 10, 13] and the very low rate (<15%) of duodenal ulcer disease [14–16] in H. pylori–infected children suggests that cagA status is not an important factor in ulcer development. I postulate that a long-term investigation of a cohort of H. pylori–infected asymptomatic children may help to resolve this enigma.

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References


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Reply

To the Editor—We thank Dr. Elitsur [1] for the comments on our study [2], and we would like to make some further observations. It seems well accepted, on the basis of the studies reported so far, that the prevalence of Helicobacter pylori infection by cagA-positive strains varies among populations, as do associated diseases such as duodenal ulcer. Dietary habits and the evolution of chronic gastritis, depending on the host response, may also be important in the development of other diseases—for instance, gastric carcinoma. In children, duodenal ulcer is usually less frequent, and thus, because of the small