Reviewer’s report

Title: The EPIYA-ABCC motif pattern in CagA of Helicobacter pylori is associated with peptic ulcer and gastric cancer in Mexican population

Version: 3  Date: 21 October 2014

Reviewer: Dionyssios Sgouras

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Major Compulsory Revisions

1. Authors determine H. pylori status only by PCR amplification of whole genomic DNA isolated from the gastric biopsies instead of bacterial DNA, extracted from the corresponding H. pylori isolated clinical strains. I am always very skeptical because this practice can be problematic, due to the presence of PCR amplification-inhibiting factors, which can reduce considerably the sensitivity and specificity in the PCR typing process. Authors should evaluate the effect of such potential inhibitory factors with the use of an internal positive control, i.e. SPUD. Alternatively, I would suggest that authors should provide data of congruence with an alternative method by which H. pylori patient status was assessed in their study group, such as culture, histological evaluation, CLO test, breath test, stool test etc.

2. H. pylori seroprevalence in Mexico for patients over 50yo is over 80% (Torres et al., Cancer Epidemiol Biomarkers Prev 2005;14(8):1874-7). Authors should offer an explanation of the much lower detected prevalence in their study group.

3. Authors should report levels of congruence between the two CagA PCR assays they used to determine the cagA-status. In addition, they should confirm CagA-negative status by a positive-result empty-site PCR (Akopyants et al. 1998 Mol. Microbiol., 28:37–53), in order to convincingly address the same issues with regards to the effect of potential inhibitory factors in the PCR mix (comment 1).

4. The method used to type EPIYA motifs assumes clonal uniformity, with reference to EPIYA motif diversity within an isolate, and requires bacterial genomic DNA. Authors should defend the suitability of their typing practice i.e. from total DNA of gastric biopsies since, quite often, within the same patient gastric biopsy, the presence of a mixed infection by strains differing solely in the number of EPIYA-C repeats, has been reported.

5. When attempting to associate particular virulence characteristics to a clinical situation, functionality of the particular virulence factor may be crucial to discern the true biological significance that lies beneath the plasticity of the H. pylori genome. Have authors been able to detect CagA protein by western blot in the corresponding patient serum samples or gastric biopsies?

Minor Essential Revisions

1. Line 116: reference needed, about the western-type of strains.
2. Line 242: ABB'C should be corrected to ABBC.

3. Line 248 should be corrected to read: 164 cagA-positive H. pylori patients with chronic gastritis.

4. Line 260: reference needed about CRPA motifs

5. Authors should correct the percentage of H. pylori-positive patients throughout the text. There are differences in the different sections that create confusion to the reader, i.e. line 58: 57%, line 233: 57.5%, line 295: 57.4%.

Discretionary Revisions

1. Table 2 and figure 1 account for the same data. I propose to add the corresponding percentages to Table 2 and delete figure 1 altogether, as it does not contribute to the comprehension of the results. Also in table 2, a footnote should be included in order to clarify the different frequencies with regards to the number of cases within groups of EPIYA motifs for sections 3 and 4. For example, the strains classified as ABC were found to be 148, whereas strains with 1 CagA-EPIYA-C motif are 149. So if different groups were grouped together for statistical assessment, it should be clearly defined.

2. Other studies have also demonstrated that the presence of CagA EPIYA-C motifs being an independent risk factor for gastroduodenal ulceration. Authors might want to include those too.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests