

Dear Editor,

Thank you for the thoughtful and constructive feedback for our manuscript, “Discovery of unique African *Helicobacter pylori* CagA-multimerization motif in the Dominican Republic”. We thank four reviewers for providing constructive comments to improve the original manuscript. We have revised our manuscript in response to the reviewers’ suggestion and are sending the revised manuscript. The changes made during the revision appear as blue color in the revised version. Itemized responses to each comment are also attached. We believe that we have addressed reviewers’ comments and hope that the revised manuscript is now acceptable publication in World Journal of Gastroenterology.

Sincerely yours,

Yoshio Yamaoka, M.D, Ph.D.

Department of Environmental and Preventive Medicine, Oita University Faculty of Medicine, 1-1
Idaigaoka, Hasama-machi, Yufu-City, Oita 879-5593, Japan

E-mail: yyamaoka@oita-u.ac.jp

Responses for Reviewer #1:

Helicobacter pylori is an important factor of peptic ulcer disease and gastric cancer. *H. pylori* produces multiple virulence factors, including *cagA* (cytotoxin-associated gene) and *vacA* (vacuolating cytotoxin), both of which are associated with increase in the risk of disease development. Authors explored sequence characteristics of the *cagA*-positive strains of *H. pylori*, according to their EPIYA patters and CM motifs in the Dominican Republic patients and found unique *cagA* CM motif in Western-type CagA. Although the subject of the manuscript is interesting and the manuscript provides detailed explanation about the molecular examination, the manuscript does not provide sufficient information about the patients, samples, groups, histological results to enable the reader critical evaluation of their study and potential value of this discovery. Namely, because only a small proportion of all infected individuals develop gastric cancer and because those with duodenal ulcer seem to be protected, it has been postulated that certain circumstances might modify the relationship between *H. pylori* infection and gastric cancer, including the genetic and phenotypic characteristics of the infecting strain *H. pylori* (*cagA* and *vacA*). To improve the manuscript I strongly suggest - Improvement of introduction section and highlight *H. pylori* infection in association with the disease development and complications - Improvement of the methods section and include briefly all necessary data about the analyses – referring to references are not acceptable – include also how endoscopic sampling for histology analysis was done (antrum, corpus, noncancerous, peptic ulcer etc. In the current version of the manuscript it is very confusing what was actually done. First authors mention that samples were taken from 258 dyspeptic patients during endoscopy (line 206-208) and were than histologically examined and evaluated according to Sydney system (line 211-214). Then authors wrote that peptic ulcer and gastric cancer were identified via endoscopy and that gastritis was diagnosed in the absence of peptic ulcer or gastric malignancy (line 214-216). What did authors mean by that?

Thank you very much for the comments. We improved the introduction by adding the association between the disease and *H. pylori* (lines 137-144). We added detailed explanations of “patient information”, “endoscopy” and “histological examination” in Material and Methods (lines 214-231). We also added results for clinical symptoms (lines 277-279).

For *H. pylori* culture, antral biopsy specimens were taken (line216) and 120 *H. pylori* strains were obtained. Then ethnicity of 120 patients was examined (self-assessment) and included in the manuscript. - Results section have only two sentences about the histological/endoscopic results e.g. “the prevalence of *H. pylori* infection determined via histological examination was 67,8% (175/258). From 258 dyspeptic patients, a total of 120 strains were isolated (120/258, 46,5%). Of these, 93 stains were isolated from subjects with chronic gastritis, 26 from peptic ulcer subjects, and one from a gastric cancer subject.” (line261-266). Discussion section again does not include information about the *H. pylori* involvement into gastric diseases and problems, instead it discusses about the heterogeneity of

EPIYA patters and CM motifs in the Dominican Republic patients in regard to their origin and ancestry – very similar what the authors already published recently in the article titled Comparative study between *Helicobacter pylori* and host human genetics in the Dominican Republic [PMID: 31675915 DOI: 10.1186/s12862-019-1526-9]. Moreover, authors stated in the manuscript that “We have previously reported that all 64 *H. pylori* strains isolated in 2011 in the Dominican Republic carried the Western *cagA* specific sequences [13]. However, this report did not mention the CM motif. In the subsequent study in the Dominican Republic, we have already performed a multi locus sequence typing (MLST) analysis of seven housekeeping genes for a total of 119 *H. pylori* strains, which were collected in 2011 and 2016, followed by population structure analysis using STRUCTURE software[14]” (line 188-194). I find their already published article [PMID: 31675915 DOI: 10.1186/s12862-019-1526-9] very interesting, while this manuscript is much less, especially because the value of the results seem to be very low and it was not discussed by authors nor addressed throughout the whole manuscript.

Thank you very much for the comments, and we are also happy that this reviewer read our previous paper with interest. This new paper is actually the expands of our previous study; however, we focus on CagA and could include many novel data and findings. We have changed the discussion by incorporating “African enigma” (lines 81–83, 121-123, 137-145, 523-524, 544).

Responses for Reviewer #2:

I read the manuscript named “Discovery of unique African *Helicobacter pylori* CagA-multimerization motif in the Dominican Republic”. (Manuscript NO: 58023) and my recommendations are as follows. Title: It is accurately reflects the major topic and contents of the study. Abstract: Adequate, summarizing the topic. Topics has been discussed with all aspects. References: References are appropriate and updated. Figures and tables are reflects the major findings of the study, and they are appropriately. This study is clearly presented. This manuscript gives additional new knowledge to the existing literature. I think that this manuscript is excellent and worthy of publication in the World Journal of Gastroenterology.

Thank you for your positive comments.

Responses for Reviewer #3:

The studies presented in this manuscript, MS No 58023, report on the distribution of EPIYA pattern and CagA-multimerization (CM) motifs associated with carcinogenic potential of Hp in Dominican Republic. Analysis of 120 Hp strains, obtained from gastric mucosal biopsies of 258 patients, revealed the presence of the unique African (hpAfrica1-type) CagA motif in Western-type CagA (Africa1-CM). However, there was no significant association between the CM motif patterns and histological scores and clinical outcomes.

While the presented experimental data clearly support the reached conclusion, the manuscript suffers from the diffused discussion, which requires judicious shortening. The same applies to the abstract.

We have changed the discussion by incorporating “African enigma” (lines 81–83, 121-123, 137-145, 523-524, 544).

Responses for Reviewer #4:

well written manuscript. I have few suggestions.

1-what is the statistical method?

The statistical method is described in line 268-271.

2-(<https://doi.org/10.1111/tbj.13174>) and (Turkiye Klinikleri J Gen Surg-Special Topics. 2018;11(2):112-4) I suggest both of these uptodate studies for the references.

The paper (<https://doi.org/10.1111/tbj.13174>) is a case report entitled “Spontaneous milk fistula from an axillary accessory breast” and Turkiye Klinikleri J Gen Surg-Special Topics. 2018; Since the paper in 11 (2): 112-4 entitled “Approaches to Recurrent/Complicated and Acute Cases” written by Turkish language. Therefore, we think we do not need to add the references in this manuscript. We hope the reviewer can understand our decision.

Responses for Science editor:

5 Issues raised: (1) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s); (2) I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor; and (3) I found the authors did not write the “article highlight” section. Please write the “article highlights” section at the end of the main text.

We submit the PowerPoint with the figure attached.

We added Article Highlights (lines 550 - 583).