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"JÚLIO DE MESQUITA FILHO"
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Title: *TLR9* polymorphisms and *Helicobacter pylori* influence gene expression and risk of gastric carcinogenesis in Brazilian population.

Authors: Manoela Dias Susi, Caroline de Matos Lourenço, Lucas Trevizani Rasmussen, Spencer Luis Marques Payão, Ana Flávia Teixeira Rossi, Ana Elizabete Silva, Juliana Garcia de Oliveira-Cucolo.

Number ID: 47439

Ruo-Yu Ma,
Science Editor,
Editorial Office World Journal of Gastrointestinal Oncology
Baishideng Publishing Group Inc

Dear Editor,

Due to our interest in publishing this manuscript (Manuscript NO: 47439) for free in *World Journal of Gastrointestinal Oncology* (*WJGO*), we are now resubmitting a revised version of the manuscript and our response to the editor's and the reviewers' comments. The changes made to the manuscript have been highlighted in yellow. We are very thankful for the comprehensive review performed to our manuscript referred above. Thus, acknowledge that the reviewers' critiques were pertinent and contributed to improve our study.

If there is any other suggestions and corrections please also forward to the responsible author Dr. Juliana Garcia de Oliveira-Cucolo (juliana.cucolo@gmail.com) to ensure prompt response.

REVIEWER 1 (18-04-2019):

Specific-Comments for the authors

In this study, the authors found that TLR9-1237 TC + CC and TLR9-1486 TC + TT, if alone or in combination, were associated with risk for gastric carcinogenesis in the Brazilian population. The presence of polymorphisms in TLR9 increases the risk for the development of gastric cancer and this issue is still controversial due to the results observed in different populations, so this study provides information on this subject

Comments:

1.- Title: a suggestion, I believe that the title should contain the type of population studied, that highlights the importance of their work.

Answer: The recommendation was accepted and we added the type of population in the title, which was as: ***TLR9* polymorphisms and *Helicobacter pylori* influence gene expression and risk of gastric carcinogenesis in Brazilian population.**

2.- Within the justification of this work. Why you just decided to study polymorphisms 1237 T/C, rs57433836 and 1486 C/T, rs187084 and did not consider the 2848 C/T, rs352140 for example? Why not explore this last polymorphism in your population? Will it only have importance in Caucasians? Check this paper: The TLR9 Gene Polymorphisms and the Risk of Cancer: Evidence from a Meta-Analysis Lu Shun Zhang, Hao Jie Qin, Xuan Guan, Kui Zhang, Zhi Rong Liu However, in the Mexican population the 2848A allele of TLR9 was more frequent in duodenal ulcer and showed an association of risk with this pathology. It would be interesting to consider it or comment on why it was not considered. Trejo de la O et al., 2015. Polymorphisms in TLR9 but not in TLR5 increase the risk for duodenal ulcer and alter cytokine expression in the gastric mucosa

Answer: We understand the issues raised by the reviewer and read the suggested articles. The polymorphisms analyzed in thus study were chosen based on bibliographical research considering studies with toll-like receptors on different types of cancers in the world population (https://www.ncbi.nlm.nih.gov/pubmed/). We then selected two polymorphisms with a potential association with risk of gastric cancer. At the time it was not possible to evaluate a larger number of SNPs due to the financial constraint and the time available for finalizing the project. We also considered the analysis of the polymorphism TLR9 2848 C/T (rs352140) in future research in our laboratory, which may enrich the SNPs results for the Brazilian population. The articles cited by reviewer were analyzed and added in the Discussion (page 13), thus being relevant and contributed to improve our results.

3.- What questions have this study to do next? 4.- What are the future directions of this work?

Answer: Considering that most cases of the gastric cancer has a good prognosis and is treatable when diagnosed at an early stage, it is of the utmost importance to establish molecular markers capable of identifying risk groups and providing early diagnosis in individuals with increased risk of developing this neoplasm. Overall, our results indicate that the TLR9 gene plays an important role in gastric carcinogenesis, highlighting the importance of the TLR9-1237 T/C-(rs5743836) polymorphism in increasing gene expression and *H. pylori* infection, possibly triggering a stronger inflammatory response, which in turn enhances the risk of tumor progression. In the future, it would importantly to investigate another polymorphism in the TLR9 gene (TLR9 2848 C/T - rs352140), described in the literature associated with cancer, but not yet analyzed in our Brazilian population.

REVIEWER 2 (25-04-2019):

Specific-Comments for the authors

Authors evaluated two SNPs of TLR9 whether they contribute to the risk of gastric carcinogenesis. The study is well conducted and the manuscript is well written and discussed. I think this article is contribute to the literature.

Answer: We thank the reviewer for their comments.

SPECIAL COMMENTS FROM THE EDITOR (25-07-2019):

Comments 1. Please upload the manuscript in word format.

Answer: The 47439-Manuscript reviewed file was submitted in word format as requested. The revised version of the manuscript and our response to the editor's and the reviewers' comments have been highlighted in yellow.

Comments 2. Please upload the Approved Grant Application Form(s) or Funding Agency Copy of any Approval Document(s) in correct format.

Answer: This study was Supported by São Paulo Research Foundation [FAPESP, grants number 2013/14022-6 and 2014/17716-1]. A screen print of the Funding Foundation's website was performed showing that all contracts were signed (http://www.fapesp.br/sage/) and the contracts and funding amounts are attached as 47439-Approved Grant Application Form (s).) or Funding Agency Copy.

Comments 3. Please provide the decomposable figure of all the figures, whose parts are all movable and editable, organize them into a PowerPoint file, and submit as "Manuscript No. - image files.ppt" on the system. Make sure that the layers in the PPT file are fully editable. For figures, use distinct colors with comparable visibility and consider colorblind individuals by avoiding the use of red and green for contrast.

Answer: The figures were decomposable according to the instructional rules and are saved in a ppt file (Manuscript no. 47439 imagefiles.ppt). We are available if there are more changes to be made.

Comments 4. Please distinguish between the title of the article series. Three levels of subtitles are allowed: (1) First subtitle: All in bold and capital, (2) Second subtitle: All in bold and Italic, and (3) Third subtitle: all in bold.

Answer: the manuscript has been revised.

Comments 5. State on the title page of the manuscript that the guidelines of the STROBE Statement have been adopted.

Answer: The STROBE statement has been added, and highlighted in yellow in the revised manuscript.

Comments 6. Please check and confirm that there are no repeated references! Please correct all cited references number like [number], then keep them superscript. Please add PubMed citation numbers (PMID NOT PMCID) and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI. PMID (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed) (Please begin with PMID:) DOI (http://www.crossref.org/SimpleTextQuery/) (Please begin with DOI: 10.**).

Answer: All references were checked and revised. The numbers DOI and PMID have been confer.

Yours sincerely,

Juliana García de Oliveira-Cucolo

Responsible Author