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World Journal of Gastroenterology

7901 Stoneridge Drive, Suite 501 Pleasanton, CA 94588, USA

Subject: Submission of revised manuscript **"** ***Stool mutations in gastric and colorectal neoplasia patients by next generation sequencing*****".** Manuscript number (36519) as a basic study article in ***World Journal of Gastroenterology***

Dear Prof. Damián García-Olmo, Prof. Stephen C Strom, and Prof. Andrzej S Tarnawski,

We thank you for reviewing our manuscript and for the valuable comments by the reviewers. We appreciate their recommendations and have taken them into consideration and modified the manuscript accordingly.

A detailed point-wise answers to reviewers' comments and the corresponding modifications to the document are described below, all changes to the manuscript are highlighted in yellow.

We are submitting the revised manuscript and hope that it is now suitable for publication.

Sincerely,

Sakari Knuutila, Professor, Ph.D.

Dept. of Pathology (CMG group),

Faculty of Medicine, University of Helsinki,

Haartmaninkatu 4, P.O.Box 105, FI-00029 HUCH, Finland.

email: sakari.knuutila@helsinki.fi

Phone: +358 504482797

Fax: +358 294126700.

2 Peer-review report

Reviewer #1:

This paper on genetic mutation detected in stool specimens from gastrointestinal tumors is rather interesting. Following are the major problems with this manuscript:

1. Change the title. It is not mutation of stool, but genetic mutation in stool specimens.

We agree with the reviewer and have now changed the title to "Gene mutations in stool from gastric and colorectal neoplasia patients by next generation sequencing".

2. Methods. Number of volunteers is low; only 14 compared to the study group of 87.

It is crucial point, and thank you for drawing attention to it. Actually the number of patients in tumor subgroups (intestinal and diffuse in stomach, and colon and rectal in colorectal) as well as the benign tumors group, ranged from 3 – 21 for each group. So the number of the controls are quite close to the number of patients in each tumor subgroup.

3. No details of the stage of malignancies are provided in the patient group.

According to the recommendations of the reviewer, we have now added details of the stage of malignancies to Table 2 under separate column "TNM staging".

4. If the malignancies are all in the advanced stage finding mutations in the stool will not be helpful for early detection.

We have added TNM staging information to the Table 2, and it is clear that among patients with mutations in stool samples six patients were in early stage of malignancy (stage I & II). Moreover mutations were also seen in patient with gastric dysplasia, as well as from patients with benign colorectal adenoma. We also added this information in the first paragraph of the discussion.

5. Age of the volunteers in two reported subjects is under 40. This is not comparable to the advanced age group in the cancer group.

We agree that this is an important point. The criteria for selecting controls was that, we wanted to have controls from healthy subjects with minimal risk of unidentified malignant or premalignant lesions. Thus the age of the controls was lower than the patients.

6. Age and number of volunteers should be comparable to the study group.

As explained response to question 2 and 5, the selection of controls was so as to avoid presence of any unidentified malignant or premalignant lesions, which is responsible for a lower age of controls. Our number of the control cases was comparable to the number of the patients in each tumor subgroup.

7. Change or add table giving the details of patients with the stage of their cancer.

Added a separate column in Table 2, indicating TNM staging for each patient.

Reviewer #2:

This is a very interesting article with regard to potential detection of stool mutations in gastric and colorectal neoplasia patients by next generation sequencing. The refore, the article should be accepted for publication under minor revisions.

1. In the Introduction section additional discrimination of mutations among gastric and colorectal cancer patients is required. Grammatical errors should be corrected. The aim of the study is clear.

We have now added information regarding discrimination of mutations among gastric and colorectal malignancies in the first paragraph of the introduction. Grammatical errors have now been corrected.

2. In the Methods section epidemiological data should be provided in the First paragraph. The section of next generation sequencing (NGS) should be better written.

Epidemiological data of the patients during the period of sample collection is now added to the first paragraph of Materials and Methods section.

We have modified the section: “next generation sequencing (NGS) methodology” to make it clearer.

In the Discussion section the structure should be re-organised.

We have re-organized the discussion part as suggested by the reviewer.

3. The Information provided in the Tables should be reduced.

We removed two columns from Table 2, which were not so important to reduce the information.

5. Newly published articles should also be included.

Added references of newly published references relevant to the study in the Introduction and in the Discussion sections.