Letter in Response to REVIEWERS

February 24, 2017

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 32537-review.doc).

Title: Mutations of the HCV NS5A Region in CHC-1 Patients Non Responders to Two or More Treatments and Its Relationship with Response to New Treatment

Authors: Paloma Muñoz de Rueda, José Manuel Fuentes Rodríguez, Rosa Quiles Pérez, Ana Gila Medina, Ana Belén Martín Álvarez, Jorge Casado Ruíz, Ángeles Ruíz Extremera, Javier Salmerón

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript No.: 32537

Thank you very much for your kind e-mail, which gave us the chance to revise our manuscript mentioned below. We have tried to emend the paper according to the reviewers' comments. I hope this revision will make our manuscript better to be accepted in your journal. The point-by point responses to the comment suggested by the reviewer are described on separate page. Each text has been altered was highlighted in bolt in the revised manuscript.

We hope that the revised version will fulfill the requirements for publication in the ***World Journal of Gastroenterology*** and give you satisfaction.

Thank you very much.

Reply to Editorial comments

Please provide language a certificate letter from a professional English language editing company. For manuscripts submitted by non-native speakers of English, please provide a language certificate from one of the professional English language editing companies mentioned in 'The Revision Policies of BPG for Article.

Answer: As non-native speakers, we have now sent the language certificate of proof-reading in the submitted documents.

The title must be informative, specific, and brief (Title should be no more than 10~12 words/60 bytes. Please revise it). Words should be chosen carefully for retrieval purposes. All nonfunctional words should be deleted, such as 'the', 'studies on', 'observations of', and 'roles of', etc.

Answer: This comment has been addressed in the revised manuscript.

An informative, structured abstract of no less than 246 words should accompany each original article. The Abstract will be structured into the following sections and adhering to the word count thresholds indicated in parentheses:

AIM (no more than 20 words): The purpose of the study should be stated clearly and with no or minimal background information, following the format of: "To investigate/study/determine…"

METHODS (no less than 80 words): You should present the materials and methods used for all of the data presented in the proceeding Results section of the abstract.

RESULTS (no less than 120 words): You should present P values where appropriate. You must provide relevant data to illustrate how the statistical values were obtained, e.g. 6.92 ± 3.86 vs 3.61 ± 1.67, $P < 0.001$.

CONCLUSION (no more than 26 words): You should present your findings and implications that are within the scope of the data you have presented in the preceding Results section. The conclusion should be written in the present tense.

Answer: This comment has been addressed in the revised manuscript.

Please read the core tip then provide the audio core tip: Acceptable file formats: .mp3, .wav, or .aiff Maximum file size: 10 MB To achieve the best quality, don't allow to have the noise.

Answer: We have now recorded the Core Tip. The Core Tip has been uploaded with the revised manuscript.

COMMENTS

Background

To concisely and accurately summarize the related background of the article and to enable the readers to gain some basic knowledge relevant to the article, thus helping them better understand the significance of the article.

Research frontiers

To briefly introduce the hotspots or important areas in the research field related to the article.

Innovations and breakthroughs

To summarize and emphasize the differences, particularly the advances, achievements, innovations and breakthroughs, from the other related or similar articles so as to allow the readers to catch up the major points of the article.

Applications

To summarize the actual application values, the implications for further application and modification, or the perspectives of future application of the article.

Terminology

To concisely and accurately describe, define or explain the specific, unique terms that are not familiar to majority of the readers, but are essential for the readers to understand the article.

Peer- review

To provide the comments from peer reviewers that most represent the characteristics, values and significance of the article, and allow the readers to have an objective point of view toward the article.

Answer: This comment has been addressed in the revised manuscript.

For the figures, the fonts and lines can be edited or moved. It can be made by ppt.

Please provide one total title. For example, Figure 1 Pathological changes of atrophic gastritis tissue before and after treatment. A: …; B: …; C: …; D: …; E: …; F: ….

Please check across the text. Please list and define all abbreviation appearing in the tables or figures.

Answer: This comment has been addressed in the revised manuscript.

Reply to reviewer's comments

The manuscript has been revised in accordance with the reviewers' suggestions:

(1) Reviewer 00052887:

"1. In which patients did you check for mutations, how did you choose them/"

The study of mutations was performed in our study cohort, selecting the patients who met the following inclusion criteria: genotype 1/4 HCV, two or more anti-HCV treatments, samples stored at -80ºC. A total of 72 patients with a stored blood sample were included in the study, having received a total of 201 treatments. HCV genomes were analysed in serum samples obtained before the patients were subjected to treatment with different antiviral therapies. The baseline serum samples of 201 treatments were amplified; samples that failed long RT-PCR amplification were excluded (n=16).

"2. Were patients chosen from all groups of therapy ?"

The 72 patients who met the inclusion criteria had received different treatments, in parallel with the evolution of HCV treatment over the years. Therefore, the patients included had baseline samples from different treatment groups.

"3. Which DAA's did you choose to treat patients?"

In Spain, treatment with DAAs (specifically, Boceprevir and Telaprevir) did not begin until late 2013. Taking into account that the patients in our study were non-responders to 2 or more antiviral treatments, genotype 1 and with an advanced stage of disease, and therefore very difficult to treat, in the time interval of our study, from 1991 to 2015, few patients were treated with first-generation DAAs; they are currently awaiting new interferon-free therapies.

(2)Reviewer by 00070056:

"1. The language and sentence pattern of this manuscript should be polished to become more accessible, many sentence and paragraph should be simplified. The word "evolution" in the title of this manuscript is inappropriate, for this research just evaluated the significance of mutation number in the antiviral treatment, it hadn't evaluated evolution and progression in cohort's genome. In addition, some errors and confusing expression are presented in the article. For example, page 7 (part of RT-PCR and direct sequencing), is it proper that viral RNA was extracted from 300 mL serum? (maybe the volume of serum is 300 μl). Page 8 (the same part), the second-round PCR product was resuspended in 20 mL and 2-5mL of it was sequenced, please check whether these numbers are regular."

This comment has been addressed in the revised manuscript.

"2. Following points haven't been illustrated well:

2.1 The method to select 72 patients.

The patients were selected in accordance with the following inclusion criteria, as stipulated on page 6 of the manuscript:

1. Genotype 1/4 HCV

2. Two or more anti-HCV treatments

3. Blood samples stored at -80°C

2.2 in page 14，"we conclude that viral variability increases when the PKRBD and ISDR regions present ≥2 mutations.", the base mutations not always induce the phenotype of virus.

We are grateful for this comment. However, the exact expression used in our manuscript is, "Taking into account the findings of previous studies [22,23,30], we considered the viral variability to increase when the PKRBD and ISDR regions present ≥2 mutations".

22. El-Shamy A, Shoji I, Saito T, Watanabe H, Ide YH, Deng L, Kawata S, Hotta H. Sequence heterogeneity of NS5A and core proteins of hepatitis C virus and virological responses to pegylated-interferon/ribavirin combination therapy. Microbiol Immunol 2011; 55(6):418-26 [PMID: 21371092 DOI: 10.1111/j.1348-0421.2011.00331.x]

23. Hayashi K, Katano Y, Ishigami M, Itoh A, Hirooka Y, Nakano I, Urano F, Yoshioka K, Toyoda H, Kumada T, Goto H. Mutations in the core and NS5A region of hepatitis C virus genotype 1b and correlation with response to pegylated-interferon-alpha 2b and ribavirin combination therapy. J Viral Hepat 2011; 18(4):280-6 [PMID: 20367792 DOI: 10.1111/j.1365-2893.2010.01305.x]

30. Nakagawa M, Sakamoto N, Ueyama M, Mogushi K, Nagaie S, Itsui Y, Azuma S, Kakinuma S, Tanaka H, Enomoto N, Watanabe M. Mutations in the interferon sensitivity determining region and virological response to combination therapy with pegylated-interferon alpha 2b plus ribavirin in patients with chronic hepatitis C-1b infection. J Gastroenterol 2010; 45(6):656-65 [PMID: 20112032 DOI: 10.1007/s00535-009-0195-7]

2.3 The critical value of baseline viral load is 600000 IU/mL，why and how to choose this cut-off value？

In Spain, until the end of 2014, quantitative tests were used to measure the amount of HCV in blood and to decide which type of treatment would be most effective, depending on the results (measured as IU/mL). The cut-off value of 600,000 IU/mL was selected by national consensus in Spain, such that patients with a viral load above this value were considered to present a high viral load, while those with lower values were considered to present a low viral load.

2.4 In Discussion, authors should discuss the potential reasons about high viral load tends to possess more mutation numbers.

This comment has been addressed in the revised manuscript.

2.5 Page 11, the diagnostic value of number of mutations within PKRBD and ISDR would be better to be presented in the form of ROC curves".

This comment has been addressed in the revised manuscript.

(3)Reviewer 03293797:

"This manuscript is a single center, retrospective observational study of genetic evolution of HCV genotype I in patients who received antiviral therapies, but were non-responders to prior therapies. The major strength of this manuscript are its relevance to contemporary practice. It could be applied to clinical judgement of therapeutic strategies in non-responder who received novel DAAs."

Thanks for your comments.

Thank you again for evaluating our manuscript and we hope it is now considered suitable for publication in *World Journal of Gastroenterology.*

Yours sincerely,

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