October 21, 2014

Editorial Office
World Journal of Gastroenterology

Dear Editor,

I, along with my co-authors, would like to ask you to consider our revised manuscript entitled **"Hepcidin/ferroportin expression levels involve efficacy of pegylated-interferon plus ribavirin in HCV-infected liver"** (Manuscript ID: 13835) for publication in ***World Journal of Gastroenterology*** as an original research article (**Invitation to Contribute Manuscripts**).

All study participants provided informed consent, and the study design was approved by an ethics review board. We feel that findings from this study will be of special interest to the readers of ***World Journal of Gastroenterology***.

We have revised the manuscript according to the comments of reviewers, provided answers to their questions, and included an outline of the revisions made.

This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have read the manuscript and have approved this submission. Financial support for this study was provided by the Research Program of Intractable Disease provided by the Ministry of Health, Labor, and Welfare of Japan and a Grant-in-Aid for Clinical Research from the National Hospital Organization of Japan. The authors report no conflicts of interest.

The manuscript has been carefully reviewed by an experienced medical editor whose first language is English and who is specialized in the editing of papers written by physicians and scientists whose native language is not English.

I shall look forward to hearing from you at your earliest convenience.
Yours sincerely,

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Answers to the Reviewers

Reviewer No. 8874

1. Figure 3A: Please explain the Hep-20 and Hep-25.

Ans.: Hepcidin exists in three isoforms, the iron bioactive 25-amino acid peptide (Hep-25) and its two amino-terminal truncated isoforms (Hep-20 and -22). In mass spectrometry-based studies, Hep-25 and Hep-20 can be measured in serum, while Hep-22 is found only in urine. [Patients and Methods, page 12, lines 4-7; Reference #42]

Reviewer No. 2528284

1. All figure legends need a more detailed description.

Ans.: We rewrote the figure legends. [Figure Legends, page 30]

2. Please add molecular weight markers in Fig. 3B.

Ans.: Molecular weight markers were added. [Fig. 3B]

3. Please provide a more detailed section material and method about the antibodies used in the study.

Ans.: Information of antibodies used in this study was added. [Patients and Methods, page 11, lines 11-15]

4. How the authors have measured IRP1 and IRP2?

Ans.: IRP1 and IRP2 were measured by real-time RT-PCR. Methods of real-time RT-PCR and primer sets are provided. [Patients and Methods, page 11, lines 4-5; Table 2]

Reviewer No. 503590

1. The choice of housekeeping gene is a bit curious. I could not find an evaluation of the stability of this gene as a housekeeping gene in the present context – if a reference exists please add, or present the evaluation.

Ans.: In each study, we have tested some genes as control genes, and selected the best one. In this study, RBBP6 was selected as well as previous studies (reference #37 and #39). [Patients and Methods, page 11, lines 4-5; Table 2]

2. I recommend analyzing effects of gene regulation on SVR in several subgroups in a multiple regression model (preferably logistic regression) in order to detect independent effects and estimate effect size as well as to avoid type I error by mass significance. This approach also allows correction for common confounders like age, sex, HCV genotype, etc. Alternatively, authors must apply a corrected critical P value (e.g. Bonferroni correction) to avoid type I error by multiple testing.

Ans.: For detecting factors contributing to SVR, multivariate statistics have been done. Although stepwise

procedure in logistic regression analysis was examined in parameters such as HCV genotype, age, gender, ALT, GGT, and IL-28B, no significant factors relating to SVR were found both in total population and in each genotype (data not shown). It may be because sample size is small for this analysis.

3. Similarly, I recommend analyzing the regulation of hepcidin gene transcription by multiple linear regression in order to explore independent effects of the proposed regulator genes as well as implementing confounder corrections.
Ans.: Also in the analysis described above, no factors regulating the hepcidine expression were detected (data not shown).

Minor comments:
4. Title: please consider to avoid abbreviations in title (*pegylated interferon; *ribavirin).
Ans.: Abbreviated words were rewritten into full spelling (Title should be less than 12 words). [Title, page 1]

5. Abbreviations: RT-PCR is brief for *Reverse Transcript Polymerase Chain Reaction
Ans.: The spelling error was transcribed into correct one. [page 10, lines 2-3]

6. How did the authors calculate the difference in gene expression? Please add this in “methods”.
Ans.: Mann–Whitney U test was adopted. [page 12, lines 11-12]

7. Table 1: please state which type of values are reported (mean? median? +/- SEM? SE? 1.96XSEM?)
Ans.: Measured values are shown in means ± SDs. [Table 1]

8. Table 1: As local variations in population and lab technology may apply, please state the normal range for each analysis.
Ans.: Normal range is presented. [Table 1]

9. Do the authors have any data on how the patients contracted HCV?
Ans.: The data are not known in all patients.

Response to the Editor’s comments

1. Title should be no more than 10~12 words/60 bytes. Please revise it.
Ans.: Title is truncated within 12 words. [Title, page 1]

2. Structured abstracts of no less than 246 words should accompany each paper. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the

purpose should be included. Please write the aim in the form: "To investigate/study/…; METHODS (no less than 80 words); RESULTS (no less than 120 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Ans.: Abstract is rewritten and word number of each section is appropriate. [Abstract, page 4]

3. **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.

Ans.: COMMENTS section is prepared according to the editor's suggestion. [Comments, page 18]

4. Please add PubMed citation numbers 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.

Ans.: All authors are listed. [Reference #38]

5. For the figures, decomposable figures are required. It means that 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. Please check across the text.

Ans.: Presentation style of Figure Legends are rewritten. [Fugure legends; Figure 2 and 3, p30]