February 20, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9013-revised.doc).

Title: Expression of P450 and nuclear receptors in normal and end-stage Chinese livers

Author: Hong Chen, Zhong-Yang Shen, Wang Xu, Tie-Yan Fan, Jun Li, Yuan-Fu Lu, Ming-Liang Cheng, Jie Liu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9013

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer #1 (02540514)

Chen and coworkers investigated the regulation of CYP isoforms mRNA and their respective nuclear receptor mRNA in diseased livers with different diagnoses. The authors conducted this study to present the first data from a chinese patient group. I can understand that this is a good intention for a study, but there are several limitations of the present study regarding the methods (mRNA determination only) and the patient number (too low, given the many different diseases).

Thanks for the general positive comments

Minor comments: No line numbers throughout the manuscript!

1.Abstract line 1: "was" instead of "were"

Corrected.

2. page 5, methods: "Severe"

Corrected

3. It remains unclear, where the normal liver probes come from and why they were obtained.

The same human CYP probes were used for both normal and the end-stage livers

4. page 11, discussion, line 2: Omit "and"

In “patient #15, #17, and #21”, we think “and” is needed.

5. page 11 + 12, discussion: Many speculations here are unsupported by the data and should be omitted. Major remarks: 1. Only mRNA data were investigated. This reduces the value of the study enormously. Western Blotting should be added at least for the central CYP isoforms and the respective nuclear receptors in order to confirm the suggested effects.

Thanks for the comments and we will perform both mRNA and Westerns in future studies.

2. From the data it is very clear that there is differential regulation independent from the cited nuclear receptors.

The authors speculate about possible explanations but it would be helpful to add data about other regulatory elements. This comprises for example cytokines (IL-1, TNFalpha) or redundant downregulating elements. Without these data this study is merely descriptive and does not add further knowledge to older studies.

Thanks for the comments and we will perform other regulatory elements in future studies.

3. The patient group is extremely heterogenous. For reliable statistical data, the diagnosis-related patient groups are too small and the whole study seems to be underpowered. The authors are encouraged to extent the study, especially in the patients with HCC. The underlying disease of the patient group with severe cirrhosis is completely unclear. The patients with alcoholic cirrhosis should be excluded (n = 5 much too low).

Thanks for the comments. However, it is not easy to get enough human samples. Although alcoholic cirrhosis samples are limited, we still prefer to include the data as the preliminary observation.

Reviewer #2 (00006071)

The manuscript by Chen et al reports changes in the expression of capital metabolic cytochrome P450 genes and nuclear receptors controlling their expression in samples of control and pathological livers obtained from a Chinese population background. The data is interesting and the methodology used appropriate.

Thanks for complimentary comments

To improve the manuscript, some changes should be introduced: Major queries: . The description of the origin of liver samples is by far too simple. The get the most from the reported data, a short description of diagnosis characteristics in order to define the liver sample as HCC, HBV cirrhosis, severe cirrhosis or alcoholic cirrhosis. Is severe cirrhosis referring to the end stage of NAFLD or to another kind of entity?

In the revised manuscript, we have added: "The diagnosis of end-stage liver diseases was made by Pathology Department of The Institute of Organ Transplantation."

Description of statistical analysis is not satisfactory. What kind of test was applied for comparison between the control and pathology group?. For comparison between peri-HCC and HCC values, a paired test was used? .

In the revised manuscript, we have added: "For the comparison between HCC and Peri-HCC, Student t-test was used".

Minor queries: . Please, correct sever cirrhosis for severe cirrhosis.

Corrected

Reviewer #3 (00038192)

Please correct typing errors like: ?sever" ?

Corrected

Total RNA was reverse-transcribed into cDNA reverse transcribed with" "than normal livers than normal livers " and grammatical errors.

Corrected

Please give patients demographics separately for the different groups.

The Table 1 is now reorganized as Disease by Gender and Age.

Please give orientation of primers in table 2

The primer is always written from **5'-3'**. We have specified "Forward" and "Reverse" in the Table 1 and this would be sufficient.

Explain fold in table 3

Provided as "-fold indicates the inter-individual variations within normal or end-stage livers".

Please define diagnosis of "severe cirrhosis"

The severe cirrhosis was diagnosed by Pathology Department of The Institute of Organ Transplantation.

"The expression CAR and CAR-regulated CYPs are shown in Fig. 1 and" this is shown in Fig. 2

Corrected

"Similarly, CYP2B6 mRNA levels in Peri-HCC were increased 53%, while in HCC decreased 40% as compared to normal livers (Fig. 2)." Should be CYP2D6.

Corrected

"Both CYP2C9 and 2C19" please add CYP "dramatic decreases in HCC by over 90% (Fig. 1)." This is figure 3.

Corrected

"bottom, CYP1A2 in 24 paired HCC and HCC-peri tissue from individual patients" is CYP2C19

Corrected

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Zhong-Yang Shen
Director of The Institute of Organ Transplant