

Dear Editor,

Please find enclosed the edited manuscript in Word format

Title: Transforming growth factor- $\beta$  and Toll-like receptor-4 polymorphisms are not associated with fibrosis in haemochromatosis

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Name of Journal: World Journal of Gastroenterology

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

- The abstract has been formatted in line with journal requirements observing specified word counts.
- A “Core Tip” has been included and conforms to journal requirements.
- The references have been altered in line with the journal requirements and redundant references have been removed, particularly in the Introduction.
- A “Comments” section has been added.
- Abbreviations used in the tables have been defined as suggested. It is assumed that the reader is familiar with commonly used scientific abbreviations such as °C.
- The Introduction has been shortened and is now more focussed with the deletion of 17 References.
- All revisions made to the manuscript appear as “underlined” text.

Revision has been made according to the suggestions of the reviewers

**Reviewer 02441729:** This study is valuable in that it includes data about both liver biopsy and DNA analysis results of C282Y homozygous patients and it is as far as I have searched the largest cohort in the literature. But a recent meta-analysis study by Wu et al. about haemochromatosis and the role of Transforming growth factor- $\beta$  genetic polymorphisms on development of liver cirrhosis has been published in Mol Biol Rep. 2013 Jan;40(1):535-43. This meta-analysis indicated that neither TGF- $\beta$  -509C>T nor +869T>C polymorphisms were associated with risk of liver cirrhosis being also in contrast with the literature like this study.

- We agree that our findings regarding concur with the results from the recent meta-analysis although the reported study by Wu et al mainly reported patients with viral hepatitis. We have now cited this paper in our discussion as it further supports that previous literature may be reporting a Type 1 error. Discussion p18 “*A recent meta-analysis considering the role of TGF- $\beta$  polymorphisms in liver disease (mainly viral hepatitis) concluded a lack of effect upon fibrosis progression in keeping with our results[70]*”

Hepatitis viruses, diabetes mellitus and steatosis are confounding factors that play important roles in the process of fibrogenesis and this study does not provide information about the status of patients by means of these conditions.

- We had previously described that this study did not include any patients with viral hepatitis and therefore this is not considered a confounding factor. Indeed the presence of viral

hepatitis was listed as an exclusion factor in designing our study. Methods, Study Subjects p9 *“Patients were excluded from this study if aged less than 16 years at the time of liver biopsy as iron loading at this age may indicate the presence of other mutations in iron homeostatic genes. Patients with viral hepatitis were excluded.”*

- We have now repeated our analyses incorporating data with regards to steatosis in those patients who had such information available. We did not observe any change in the lack of association between gene polymorphisms and fibrosis stage and have included a statement to this effect (see Results, p13 and p14). Discussion, p15 *“Analyses were repeated incorporating data on steatosis grade however this produced no statistically significant effect.”* As expected, there is an association between fibrosis and steatosis (see Table 3) which has been previously reported. Unfortunately, we cannot report on the role of diabetes as this data is not available for most patients however given that the presence of steatosis did not alter our overall conclusions, we have no reason to suspect that diabetes would and we do not feel this diminishes the study.

The introduction part is too long. Forty-five references were present but I think that more than three references are unnecessary. The authors should keep the introduction short and tell the readers why the study was done and explain why this study is important in this section.

- As suggested, the Introduction has been substantially streamlined and focussed with 17 references removed. We believe it is important to explain why the candidate genes were chosen for this study and the Introduction is the most appropriate place for this to occur.

**Reviewer 00012156:** Wood et. al., studied gene polymorphisms in TGF-b and TLR4 in the patients of haemochromatosis with hepatic fibrosis in this paper. The study is very interesting but there are some comments to contents. Major comments: 1. Introduction is too long and what did authors eager to mention in this part? The introduction should be more compact.

- We have streamlined the Introduction including removal of a total of 17 redundant references as suggested. We believe it is important to explain why the candidate genes were chosen for this study and the Introduction is the most appropriate place for this to occur. We believe that the Introduction is not more compact and more focussed.

2. Why did authors select the 8 SNPs of 6 genes i.e., TLR4, TGF-b, OGG1 etc.? There was no convincing description about the reason.

- We outlined in the introduction and methods section that we wished to examine genes involved in hepatic inflammation, signalling to hepatic stellate cells and fibrogenesis. We also highlighted that we wished to consider functionally significant polymorphisms in hepatic fibrogenesis and to replicate previous positive studies in smaller cohorts. Introduction, *“Candidate genes for analysis were chosen based either on their existing association between gene mutations and fibrogenesis in other disease aetiologies, or their demonstrated role in hepatic stellate cell biology and hepatic injury/fibrosis”* (further discussion regarding this is also found in the Methods section)

3. Authors stated that this study was carried out in the largest groups in the international literature, but in the discussion part they described that it would be required greater number of subject. The analyses of the data were not unified in this paper and most data were negative. This means the limitation of the method of this study.

- Although the data has not demonstrated a positive result, we do not feel that this represents a “limitation on the methods of this study” but in fact, suggests that our cohort size and methods are robust and that a null result is likely to be true. Although studies with positive results are more likely to be published, we felt it important to show that our data does not agree with previously published work (in smaller cohorts), and therefore the genetic influences on fibrosis progression in haemochromatosis remain unknown. This is important information.

4. In discussion part the authors mentioned TNF- $\alpha$  but there was no data of TNF- $\alpha$  in the results.

- We did not set out to study TNF- $\alpha$  and therefore have removed the reference to it.

5. From the results of this study and the references, gene polymorphisms in TGF- $\beta$  and TLR4 were not associated with a hepatic fibrosis in hereditary haemochromatosis. Data were negative but results have some meaning in the etiology.

- We agree.

**Reviewer 00227406:** No comments made by this reviewer requiring amendments to the manuscript.

**Reviewer 00012328:** No comments made by this reviewer requiring amendments to the manuscript.

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