Reviewer’s report

Title: Hepcidin gene polymorphisms and iron overload in β-thalassemia major patients refractory to iron chelating therapy

Version: 0 Date: 15 Apr 2019

Reviewer: Vasilios Berdoukas

Reviewer's report:

The authors have evaluated the ferritin levels, cardiac iron and hepatic iron in 102 patients with thalassaemia major on regular transfusion and chelation therapy, according to whether they had some upstream mutations related to their hepcidin producing gene. They evaluated 3 mutations and found homozygosity for the c.-582A>G allele to be the most common and that it was associated with more patients having excess cardiac iron. The c.-153C>T mutation was not present in their population and the c.-443C>T was only present in the heterozygous form in 10 patients. The latter mutation showed tendency towards being associated with high levels of ferritin however it did not quite reach statistical significance.

There are a number of problems with the paper. Firstly with respect to the structure, it is unusual to have the methods after the discussion. The methods should move to be above the results. The discussion starts off with a repetition of information that is in the introduction. Discussions should start with a brief description of the findings of this study and its significance. For example, it could start with "i.e our study has shown that the allele GG genotype is associated with significant degree of cardiac iron overload...." and then continue with a brief description of the other findings With respect to significance, it might be reasonable to suggest that these patients may need more intensive chelation and perhaps choosing a chelator that is better at removing cardiac iron. All the results that are in the discussion should be in the result section and could be tabulated if that saves time. The discussion should included the purported mechanisms of the greater iron load with other references. These are there but they are then associated with the results.

The authors have used Anderson et al for the evaluation of hepatic iron overload. However it was acknowledged that this method underestimated the liver iron concentration and subsequently many centres either use ferriscan or the Wood formula \[\text{Fe}R^2* = 0.0254 \times R^2* + 0.202\]. The level of LIC that is optimal is controversial however absolute normal is regarded as &lt;1.1mg/gm dry weight, this would mean an R2* of &lt;42.5 which converts to a T2* of &lt;23.5 msec. A range of 1.1 to about 3.5 would be a T2* of 23.5 to 7.7 and this would be mild, then from 3.5 to 7 would be 7.7 to 3.6 which would be moderate and then below 3.5 would be heavy iron load. Then it would be appropriate to recalculate the results according to the degree of liver iron load rather than using ferritin as the cut off. Reference 5 seems incorrect. A more appropriate reference might be Orphanet J Rare Dis. 2010 May 21;5:11. doi: 10.1186/1750-1172-5-11.Beta-thalassemia.Galanello R1, Origa R.

Line 33 of introduction - instead of acting on - including would be more appropriate. Line 39-40 should discuss the role of hepcidin on the ferroportin of macrophages and hepatocytes. This sentence and the following one could be restructured so that the flow is better.

Line 52. Hepcidin is low in the thalassaemia intermedia patients. In well transfused thalassaemia major patients it is not quite as high as in normal individuals but it seems sufficient to suppress iron absorption and release from macrophages.

At line 56-57 the discussion should include the role of erythroferrine and how it influences hepcidin

A minor point. Is not the corresponding author and author?
The patients who do not have the mutations searched for are basically the controls for this study

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published
Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal.