Reviewer’s report

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

Version: 1 Date: 10 Oct 2019

Reviewer: Vasilios Berdoukas

Reviewer's report:

The authors have attempted to respond positively to the previous review topics. There are however a number of issues that still need to be addressed. In the abstract the authors need to clarify the iron load in the heart separate from the iron load in the liver. The 70 (30% not 3%) is definitely incorrect. They need to put in the cardiac and liver separately ie approximately 60% for heart and 11% for liver. On page 7 "Details of statistical analysis have been shown in figures 1 and 2." It is not quite statistical analysis. It is just a distribution plot of the results with percentage on the y axis and the normal, .... severe or very severe in the x axis! In table 2 the patients with the GG, have lower pretransfusion Hb levels (which I believe these are), these patients may have lower hepcidin levels as their erythroferrin would be more active and more likely to induce a lower hepcidin level. Therefore, these patients need to have had hepcidin levels in order to be able to truly interpret these results. On page 7 where the authors state the following: For c.-153C>T, all samples were homozygous for allele. Does this mean that this mutation may be a polymorphism that is also a wild form of hepcidin? Needs to be discussed. Page 9 where the authors state: most of them proved to be refractory which can (should read "may") be attributed to different above-mentioned genetic variations. Should add the following: or to lack of acceptance of their chelation therapy or inadequate availability of the chelation. Minor point: Page 10 Different racial (insert characteristics) may explain the discrepancy between our results and others. Last line of discussion: The use of mini-hepcidin is (may be not is) an alternative to a high dose of chelating therapy in these patients.

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

No

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

No

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

No
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

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