Author’s response to reviews

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

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Author’s response to reviews:

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“BMC Medical Genetics Editorial Office”

Dear Dr. Matteo Pasini

On behalf of all authors I would like to thank you and the referees for reviewing our manuscript entitled “Hepcidin gene polymorphisms and iron overload in β-thalassemia major patients refractory to iron chelating therapy.” (MGTC-D-19-00121R1)
We have revised the manuscript according to the referees’ suggestions and we have marked all changes by highlighting them in the revised manuscript. Please find below our point by point reactions to the remarks of the reviewers.

I would be most grateful if you accelerate the reviewing process as this has been taken a long time since our first revision in the July. This manuscript is the result of an MSc student who will not graduate before she has a published paper.

Thank you for giving us the opportunity to resubmit a revised version of our manuscript. We look forward to hearing from you.

Yours Sincerely,
Majid Shahabi
Reviewer 1

1- 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.
Response: 71.3% patients were iron overloaded based on plasma ferritin >1000 ng/ml as we elaborated in the text

2- On page7 "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”.
Response: Figure 1 and 2 were deleted.

3- 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.
Response: In a study by Azarkeivan et al, they evaluated the relationship between serum hepcidin level and ferritin level in 82 thalassemia major patients, but no relationship was found. They believe hepcidin levels could not predict the severity of iron overload in the liver or cardiac in these patients.

4- On page 7 where the authors state the following: For c.-153C&gt;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.
Response: Island found only one sample to be heterozygous for this variant. We also could not find any heterozygous sample for this variant. So it could be deduced that this variant is a mutation and not a polymorphism.

5- 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.
Response: “May” was replaced. We are completely sure that chelation therapy was adequate and is followed regularly by the patients.

6- Page 10 Different racial (insert characteristics) may explain the discrepancy between our results and others.
Response: We changed the word as you mentioned.

7- 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.
Response: We changed the word as you suggested.
Reviewer 2

1- The statement "Beta-thalassemia is the most common group of hereditary hemoglobinopathies" should be replaced by "Beta-thalassemia is one of the most common group of hereditary hemoglobinopathies" in both Abstract and Background.
Response: We changed it as you mentioned.

2- Still in the Abstract, in the phrase "Several studies emphasized the role of single nucleotide polymorphisms (SNPs) located in the promoter region in the expression of the gene.", "in the expression" must be removed;
Response: It was deleted.

3- "A total of 102 adult beta-thalassemia major patients" since they are all adults;
Response: We added this word.

4- "All samples were homozygous for allele T of c.-153T&gt;C" is the opposite: all samples were homozygous for allele C (and the mutation is c.-153C&gt;T), according to the main text.
Response: It was corrected.

5- In the Background, the average frequency of beta-thalassemia in Iran, as well as its relationship to malaria, should be commented.
Response: We mentioned this sentence in the text “These are the areas that were endemic for malaria in the past”.

6- The first sentence of the next paragraph (line 23, page 3) can either be removed or should include the introns as well, as many mutations occur in these regions, including IVS-II-1
Response: This sentence was revised.

7- At the end of the Background, the absence of other similar studies in the country should be emphasized. It is the justification of the work.
Response: We added this sentence “Although several studies have been conducted on molecular genetics of thalassemia in Iran, to the best of our knowledge, this is the first molecular study of hepcin gene in β-thalassemia major in our country”.

8- In the Results, the 'p' values of the allelic and genotypic frequency analyzes for Hardy-Weinberg equilibrium evaluation should be included in Table 3.
Response: It was added.

9- The median and the range would more adequately express the serum ferritin levels in Table 2.
Response: Median (25%-75%) was added in table 2. The range for the AA/AG group is 14068 and for the GG group is 5992.

10- In the Discussion, "All patients were homozygotes for c.-153C&gt;T variant" should be replaced by "All patients were homozygotes for the normal allele (c.-153C)", Response: It was replaced.

11- At the end of the Discussion, it would again be interesting to emphasize that this is the first study of its kind in Iran and what is its relevance.
Response: It was added at the beginning of the discussion.
Reviewer 3

1. Table 2. In this table the authors compare 96 AA/AG patients with 6 GG patients, which is a very weak comparison. I suggest dividing the patients into three groups based on the c.-582A&gt;G genotype (AA: 55 patients, AG: 41 patients and GG: 6 patients) and redo the statistical analysis. If the G allele is a modifier, then heterozygotes will possibly show statistically significant differences from the other two groups. Considering the fact that the number of patients in the heterozygotes group is high, the suggested analysis will possibly further support the role of the G allele as an iron overload modifier.

Response: We divided the patients in 3 groups, but no statistical correlation was found between this variant and serum ferritin level, hepatic iron concentration and cardiac iron concentration (p value = 0.20, 0.10, 0.06 respectively). As we discussed before the major allele is “A”, we divided the genotypes in a manner that the homozygous minor allele “G” is evaluated versus major allele.

2. Remove figures 1 and 2 and the related text in the manuscript. The results presented in the figures are confusing and not described in the Results and Discussion sections.

Response: Figure 1 and 2 were deleted.

3. Line 51: replace 'iron hemostasis' with 'iron homeostasis'

Response: It was replaced.