Author’s response to reviews

Title: Molecular characterization of β-thalassemia intermedia in the West Bank, Palestine

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Version: 1 Date: 04 Jan 2019

Author’s response to reviews:

Dear Editor,

Below is a point-by-point response to reviewers’ comments. The required changes were introduced into the manuscript and highlighted in yellow, and their locations is indicated below.

Regards

Mahmoud A. Srour
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Technical Comments:

Editor Comments:

BMC Hematology operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reviewer reports:
Ahmet Emre Eskazan (Reviewer 2): I have reviewed the manuscript. This is a nicely performed study, and a well written manuscript. For my point of view, the manuscript can be accepted for publication in its current form.

Valentina Brancaleoni (Reviewer 3): The paper by Faraon et al. reports the molecular characterization of intermedia thalassemia in Palestine. The work expand the knowledge about genotypes/phenotype correlation in TI.

In some cases in the result section some paragraph are not clear (see below), since the reader have to suppose some facts that are explained in the discussion section. But I would like to understand the results immediately, and not reading the discussion. Please, try to be more clear.

Comment: It is not clear to me why it is not possible to have a TM patient older than 30 years… With the correct follow up and treatment the life expectation of TM (or better transfusion dependent) thalassemic patient is now greatly increased.

Answer: We agree with you that the life expectancy of thalassemic patients has been greatly improved and they of course can live beyond 30 years. However, in our country most thalassemia major patients die in their 20s (based on the information from the Thalassemia Patients’ Friends Society in Palestine) and this is probably due to the lack of appropriate or systematic management plan.

Comment: You report that 'most of patients were results of consanguineous marriages'. Please, report also how many homozygotes (% or number) are from consanguineous marriages in the section 'b-thalassemia genotypes'.

Answer: We have re-analyzed our data and found that from 51 TI patients, 47 (92.2%) belonged to relative parents and 4 (7.8%) belonged to non-relative parents. While in our manuscript, we reported that only 3 patients belonged to non-relative parents (page 7; lines 10-11). Now this mistake is corrected and the percentage of each group is given in same sentence (page 7; lines 10-11).

In addition, we have added a new paragraph in the “β-thalassemia genotypes” section addressing this point. The new paragraph is on page 12/ lines 18-20 and continues on page 13/ lines 1-2 and states:

Analysis of the effect of consanguineous marriages on β-thalassemia genotypes, revealed that among the 47 patients who belonged to relative parents, 44 patients had homozygote and 3 patients had compound heterozygote β-thalassemia genotypes. On contrast, of the 4 TI patients who belonged to non-relative parents, 3 patients had heterozygote and one patient had homozygote β-thalassemia genotypes.
Comment: Table 2: please check carefully table formatting, since in the provided file is not clear. It lost the line definition, since for space issue the text goes sometimes down. In some case, I did not couple correctly beta and alpha genotype.

What do you mean with (1:0:0) in the column of alpha thal? Please add a caption to explain this.

Answer: In the file that I have submitted, Table 2 is complete. B-thalassemia genotypes are shown in first column, a-thalassemia genotypes are shown in the fourth column and the column headings are correct. If I did not understand you or answer your question, please specify your inquiry.

The numbers (1:0:0) are used to emphasize that only one patient (referred to as 1) from 3 patients described in this raw, has the (α3.7/αα) genotype and his hematological values are placed at first position, respectively.

Now a caption under Table 2 is added to explain this point. The caption added states:

The numbers (1:0:0) are used to emphasize that only one patient (referred to as 1) has the (α3.7/αα) genotype and his hematological values are placed at first position, respectively.

Comment: Beta A: is it the normal beta allele? I did not understand at a first glance. I suggest to use instead 'wt' (wildtype) is more comprehensible to the largest majority of readers.

Answer: The βA has been replaced by βwt throughout the manuscript.

Page 11 line 41: (talking about group III): which patient had the occasional transfusion? The one with the coinherence of alpha 3.7 deletion? Which patient did never show splenomegaly? Always the one with 3.7 deletion? If yes, I should state it more clearly to the reader and not only let to the reader's interpretation.

Otherwise, I did not understand correctly, so please specify in both of cases.

Answer: I have re-written the respective paragraph (page 11/ lines 14-18) to clarify the points you raised. The new paragraph states:

Group III: Homozygosity for IVS-I-110 G>A. This group included three patients. One of them had α3.7/aa genotype, had regular blood transfusion and showed splenomegaly. The other two patients had normal α-genotype, one of them had regular blood transfusion and showed no symptoms of splenomegaly while the second patient had occasional transfusion and was splenectomized.
Page 11, line 46: the 'patient' homozygous for c.-151C>T, we can say that it is a silent case, since Hb is almost normal, he/she never transfused and I think there are no other symptoms. I think you detect it only for HbF % and HbA2 level. I should state this fact.

Answer: Yes, we agree with you.

Page 12 line 26: the correct spelling is 'fourth' not forth

Answer: The mistake is now corrected (page 12/line 8)

Page 12 line 39: You referred to the wrong table, did you mean table 3?

Answer: In page 12/ line 13 (first version of the manuscript), the sentence “One patient was splenectomized and one showed symptoms of splenomegaly” provided more information on a case presented in Tables 2 and 3. But the data on splenectomy, splenomegaly and frequency of blood transfusion are not given neither in Table 2 nor Table 3. Now this paragraph (including this sentence) has been rewritten per your request (see next comment) and references to Table 3 have been appropriately provided. See the answer to the next comment below.

Comment: Page 12 line from 26 to 41: which was the patient who have a better phenotype? The one with also alpha mutation? Please, explain more clearly this issue to the reader (as above).

Answer: The paragraph concerning the β0/β0 genotype has been rewritten to make it clearer to the reader. The new paragraph (page 12/ lines 10-16) now states:

However, in this group the co-inheritance of Gγ-globin gene XmnI SNP in three patients (Table 3, patients # 1 to 3) and heterozygosity for α-thalassemia (αIVSI(-5nt)α/ αα) in one patient (Table 3, patient # 4) has ameliorated the thalassemic phenotype in this group. Patient # 3 (Table 3) has the highest HbF values, was never transfused and showed no symptoms of splenomegaly. While the other 3 patients (# 1, 2, 4; Table 3) were on regular transfusion (every 2-3 months), 2 patients (# 1 and 4) showed no symptoms of splenomegaly and one patient (# 2; Table 3) was splenectomized.

Comment: Page 14 line 51: I think this sentence is not useful, you refer to table 5 in the discussion; so you can avoid it.

Answer: The sentence referring to Table 5 at the end of the results section has now been removed. Since this sentence is the only one in the results’ section that mentions Table 5, we have moved Table 5 to the discussion part just after the second paragraph, where Table 5 is first mentioned.
Comment: Although the authors are aware of the existence of other genetic modifiers influencing thalassemia phenotype, they did not clearly discuss the results citing the possible influence of other loci (BCL11A and HMIP loci) as other determinants of HbF production. I would suggest to analyze this loci in their patients if it would possible and to add to the paper. Since I understand that it is not always possible, I kindly suggest to address this issue and to insert it in the discussion section. i.e. page 21 line 14: which major QTL?

Answer: The role of genetic modifiers in ameliorating the thalassemia phenotype has been addressed in the discussion section. See page 21/ lines 16-21 and continues on page 22/lines 1-16. Additionally, the last paragraph in the discussion section (page 22/ lines 17-19) has also been re-written to be consistent with the previous paragraph. Four new references are cited in this paragraph and numbered 42 to 45.

Concerning your request to analyze the BCL11A and HBS1L-MYB (HMIP) in our patients, we believe that this part was not within the scope of our study. Additionally, in 87.3% of TI patients, the inheritance of β+ allele (IVSI-6, IVSI-110, IVSII-848 & beta-101) explains the mild phenotype of thalassemic patients. Also, we analyzed XmnI SNP and α-thalassemia which in turn explained the mild phenotype of TI in a proportion of patients. Thus, the role of QTL other than XmnI SNP and α-thalassemia (tested in this study) is expected to be limited among our study subjects.

The major QTL are XmnI SNP of γ-globin gene promoter, BCL11A and HMIP (HBS1L-MYB). The loci names are now shown after the term QTL in the discussion section (page 21/ last line).