Author’s response to reviews

Title: Molecular Analysis of a Large Novel Deletion Causing α+-Thalassemia

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Response to Reviewers

Response to Reviewer #1 (Maria de Fatima Sonati)

Reviewer #1 (Rev #1): Q1: Firstly, the text must be fully revised by a native English speaker.

Response (R): Thank you for your recommendation. This manuscript was revised thoroughly by a Native English editor, and the abstract was marked in red typeface.

Q2: In the Abstract, the specifications of the hematological analyzer and the electrophoresis equipment, as well as their results, are not necessary - the most important is to describe how the
detection and characterization of the novel deletion was carried out. It is sufficient to inform that it was an adult male patient of Chinese origin with Hb H disease. On the other hand, there is no mention of the MLPA and DNA sequencing, both used in the detection and characterization of the deletion.

R: Thank you for your advice. The abstract section has been rewritten. The procedure of detecting and characterizing novel deletion was elaborated. The methods and procedure of MLPA and DNA sequencing is also elaborated. The modifications were all marked in red typeface.

Q3: In the Background (lines 46 to 49), the authors should assume that this is a case of Hb H disease.

R: Yes, this is corrected and clarified. The modifications were marked in red typeface.

Q4: In Methods, references are missing in lines 23 (gap-PCR), 27 (PCR-RDB), 31 (common beta-thalassemia mutations in Chinese) and 42 (deletion breakpoints).

R: Yes, these references are added.

Q5: In the Results, the authors should assume that it is Hb H disease (not a suspect). If there is no relationship of consanguinity between the patient and his wife, rather than informing his hematological data, it would be important to inform those of his parents, children and siblings if possible. Was the complete familial analysis not done?

R: Thank you for your advice. We recalled the son of the proband for further testing, although the siblings and parents of the proband refused to participate in this study. As shown in the manuscript, Blood test results showed that his mcv, mch decreased, and hemoglobin electrophoresis demonstrated a decrease in HbA2. Finally, the results of the thalassemia genetic test showed that the genotype was a Southeast Asian-type thalassemia heterozygous, which was inherited from the proband. And the modifications were marked in red typeface.

Q6: Table 1 should be supplemented with the other hematological values: RBC, Hb, Hct, reticulocyte counts, etc. (does the patient have no hemolytic anemia?), as well as with family data, if possible. It needs a title.
R: Yes, the table is updated with required information. All the modifications were marked in red typeface.

Q7: Figure 2 is not necessary.
R: Yes, this is removed.

Q8: In the Discussion, references are missed; the second and third paragraphs are repeats of what is already stated in the Methods and may be summarized to a few sentences.
R: Thank you for the advice. The discussion section was revised to enhance the clarity.

Q9: Finally, the Discussion could be enriched by comparing this case with those whose Hb H disease is caused by the association of the alpha0 allele with the -alpha3.7 and alpha4.2 alleles, the most common.
R: This is a great advice. These comparisons were added to the discussion. All the modifications were marked in red typeface.

Response to Reviewer #2

Response to Reviewer #2 (Ju Long)

Reviewer #2 (Rev #2) : Q1: The English should be completed improved.
R: Yes, a native English Editor has revised this manuscript. The modifications were marked in red typeface.

Q2: Too many mistake on writing, such as:
Page 4 line 19 " toα0-Thalassemia" should be "to α0-Thalassemia"
Page 4 line 29-30, "HbConstantSpring"," HbQuongSze" and "HbWestmead" should be "Hb ConstantSpring"," Hb QuongSze" and "Hb Westmead"
Page 4 line 46 "theα-globin" should be "the α-globin"
Page 5 line 29 "Hb WS" should be "Hb Westmead"

Page 5 line 50 "5 x buffer4μL" should be "5 × buffer 4μL"

R: Yes, these are corrected and marked in red color. At the same time, the same writing errors in other places of the article were also modified and marked.

Q3: The author should rewrite the abstract.

R: Yes, the abstract was rewritten as suggested.

Q4: 3.3 Detection of rare α-Thalassemia genes. The author should list the primers used in this research. Especially, the author should clearly described which "fusion gene" the author indicated to.

R: Thank you for your advice. Six α-thalassemia genotype testing kit (Yaneng Biological technology Co., Ltd., Shenzhen) was used to detect the rare α-Thalassemia genes. All the primers come from the kit. Addition, the fusion gene we detect in that rare thalassima kit was a fusion between the α2 and ψα1 genes. The recombination began at exon 3 of α2 gene, crossing with exon 3 of the ψα1 gene. With this recombination, the conservative 3’UTR of the α2 gene was changed, and an extensive transcript with a new signal 1048 bp 3’ to the terminating codon was found, which finally caused α+-thalassemia (reference23). The reference was marked in red typeface in the Result 3.3.

Q5: Pedigree analysis should be performed. Only analyzed the proband and his wife was not enough.

R: This is a great advice. The son’s data were added to this study. As shown in the manuscript, Blood test results showed that his mcv, mch decreased, and hemoglobin electrophoresis demonstrated a decrease in HbA2. Finally, the results of the thalassemia genetic test showed that the genotype was a Southeast Asian-type thalassemia heterozygous, which was inherited from the proband.

Q6: The electrophoretic effect shown in Fig. 1 is too poor.

R: Yes, this is adjusted and revised as requested.
Additional experiments that were carried out

1. We recruit the proband's son for hematological analysis, including routine blood test and hemoglobin electrophoresis. The results showed that MCV, MCH were decreased, as well as Hb A2. Unfortunately, the brother and parents of the proband refused to conduct further testing.

2. Subsequently, we conduct conventional thalassemia gene for further detection, and the test result showed that his genotype was --SEA/aa.

3. Six α-thalassemia genotype testing kit (Yaneng Biological technology Co., Ltd., Shenzhen) was used to detect --THAI, -α27.6, HKαα, fusion gene, ααanti4.2 and ααanti3.7.

4. The primers P1 and P2 were used to detect the novel breakpoint in the proband’s son and showed no product.