Author’s response to reviews

Title: Clinical course, mutations and its functional characteristics of infantile-onset Pompe disease in Thailand

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Author’s response to reviews:

Dear the editor of BMC Medical Genetics
Re: MGTC-D-18-00480

Thank you very much for your kind consideration of our manuscript entitled “Clinical course, mutations and its functional characteristics of infantile-onset Pompe disease in Thailand”. We sincerely appreciate your valuable suggestions. All the comments we received have been taken
into account to improve the quality of the article. In the following pages are point-by-point responses to each of the questions and comments raised by the reviewer. All corresponding changes have been added to the manuscript and are highlighted in yellow.

We believe the changes made to the manuscript based on the reviewer’s comments have significantly improved the manuscript as intended and are sufficient to make our manuscript suitable for publication in BMC Medical Genetics. We look forward to receiving your reply soon.

Sincerely yours,
Duangrurdee Wattanasirichaigoon, MD
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Point-by-point response

Reviewer 1

The AA report the results concerning molecular diagnosis, follow-up and response to the ERT treatment in a Group of 12 Thailand patients with infantile-onset Pompe disease (IOPD), aged between 15 days and 8 months. All patients presented the classical disease appearance at birth characterized by hypertrophic cardiomyopathy and generalized muscle weakness. The GAA assay showed levels of the enzyme less than 1%. Interestingly, the molecular analysis showed five novel variants, validated by transfection analysis in COS-7 cells. These novel mutations were located in both N-terminal beta-sheet domain and in the catalytic domain. Furthermore two hotspot regions were identified, respectively in exons 14 an 5, Accounting for the 62% of all mutations. The manuscript is very interesting as it provide new acquisition in the field of molecular variants causing IOPD in Asia. An adding value is the possibility to limit the analysis to these two exons, as first approach sparing cost and time and speeding up the diagnosis times. Only 3/12 (25%) of the patients - sharing different mutations - received ERT therapy, at 4 weeks, 6 and 11 months respectively. Rather than the type of mutations, the different results seem to be related to the start time, earliness of treatment, so I would suggest to the authors not to emphasize in the abstract and conclusions the sentence " These data can be useful..................................and provide additional information of the genotype/phenotype correlation and prediction of long-term response to enzyme replacement therapy...." for the reasons just mentioned (small number of treated patients and the different mutations).
Response: We remove the sentence “These data can be useful........... long-term response to enzyme replacement therapy...." from the conclusion of abstract and conclusion section.

It is replaced by “These data can benefit rapid molecular diagnosis of IOPD and severity rating of the mutations can serve as a partial substitute for cross reactive immunological material (CRIM) study.” (Abstract section: Page 4, lines 76-78; conclusion section: Page 20, lines 483-485)

Reviewer 2

Clinical course, mutations and its functional characteristics of infantile-onset Pompe disease in Thailand is a well thought of manuscript with an objective of examining clinical and molecular characteristics of infantile-onset Pompe disease majorly in Thailand and in some other countries from that region. This paper would definitely add more to the knowledge of IOPD and molecular findings seen in the region there. However, there are some major and minor revisions needed to the manuscript, as below:

1. The major issue is with wrongly written GAA variant in their list of novel variants. This is a major typo mistake starting from abstract and continues into introduction; c. 2065G>A (pseudodeficiency allele) does not result in protein change p. D513G in exon 10. C.1538G>A is the correct nucleotide change here. They corrected this mis-calculation lateron in the manuscript but failed to read the mistakes in abstract and introduction section. Protein change associated with this nucleotide change is p. Glu689Lys in exon 15. Please check carefully and do the needed corrections.

Response: Abstract section: The c. 2065G>A in replaced by C.1538G>A (Page 3, line 68)

2. This variant p.513 variant may not be a novel variant, it has been seen and published before it seems. Please check again.

Response: The change of alanine at position 513 to glutamic, p.Ala513Glu, was first reported by Hermans et al. However, there is no report describing the change from alanine at this position to glycine residue, so we reported this variant as a novel one.

3. May be a good idea to write Amino acid change in 3 letter codes throughout the manuscript rather than single Amino Acid letters for clarity and easy to follow for readers. Single letter codes get hard to follow and mistakes happen.

Response: We now use three letter codes throughout the manuscript including tables and figures (except for Table 1, we still keep the code name of primers).

4. Grammatical errors in abstract and introduction (line 58 - GAA gene was analyzed). Likewise there are quite a few grammatical errors throughout the manuscript, please read it carefully.

Response: “GAA gene was analyzed”. (Page 3, line 58)

We also edit the other grammatical errors.

5. Write full word for abbreviation CRIM - where it is used the first time (abstract and background).

Response: The full word is used where it is mentioned for the first time.

- Abstract: (Page 4, line 77-78)
- Background: (Page 6, lines 130-131)

6. GAA has 20 exons but only 19 exons are coding exons (initiation codon in exon2) and get sequenced usually using in most diagnostic labs. Did the authors sequence non-coding exon 1 as well, please clarify.

Response: PCR of all exons including the noncoding exon (exon 1) was performed and subsequently subjected for Sanger sequencing. (Page 7, lines 165-166)

7. Severity rating of the mutations section - lines 216-218 - it is very important to clearly state that what protein band of GAA protein as seen on Western blot is associated with each class of severity rating; i.e what protein bands were visible or missing in Class A, B, C categorized patients - precursor bands or mature band etc. Just saying protein band present does not suffice for this rating analysis.
Response: We provide more detail explanations about how severity is rated including what band(s) is missing or visible (Pages 9-10, Lines 210-242), since this is quite detailed, making it is a bit lengthy. If the reviewer feel that it should be shorten, we will be pleased to do so as needed.

8. line 236 - Excluding the outliner - should be outlier not outliner. Likewise line 251 - it should conversation with the referring physician, not conversation of the referring physician; line 259 - should be myopathies facies not myopathies facie.

Response:
- It is now ‘outlier’ (Page 11, line 255).
- It is now ‘myopathic facies’ (Page 12, line 278).

9. Line 275 - c.1327-2A>G is IVS8 intronic splicing mutation and not an in frame deletion as depicted by authors - please check and correct and clear about it.

Response: The c.1327-2A>G is IVS8 variant is indeed a splicing mutation. It also leads to exon 9 skipping and results in an in-frame deletion of 37 amino acid residues. We edit the content accordingly. (Page 12, lines 295-296)

10. line 276 - p.Asn675del - is an in-frame deletion, please check and correct it.

Response: It is said so. (Page 12, line 296)

11. Line 280 - variants c. 1726 G>A and c. 2065G>A - are both well-known and well documented pseudodeficiency alleles and they should be written as such. Should not be written as GAA mutations.

Response: The words ‘mutations’ is replaced by ‘variants’. (Page 13, line 302)

12. Line 304 - Protein band at 76Kda is a well-documented and published mature GAA protein band along with 70Kda band. It should not be written as an intermediate band size. Most adults show both these band sizes on western blots.

Response: Thank you. We have replaced ‘the protein band at 76KDa intermediate form’ with ‘mature form. (Page 14, lines 327-328)
13. Line 307 - Have authors sequenced protein band at 36Kda to make sure that it is indeed GAA protein band. Good to do that to make sure that it is not a spurious band being picked up. There is no mention of this 36Kds band in literature for GAA protein.

Response: We did not sequence the 36KDa band but we predicted the molecular size of p.Tyr292X product (residues1-38) by calculating the molecular weight of p.Tyr292X which is approximately 32.28 KDa. This peptide also contains 2 N-linked glycosylation sites (140 and 233) which possibly results in retardation of protein mobility in SDS-PAGE as observed at the molecular size of around 36 KDa. (Page 14, line 330)

14. Discussion - line 390 - again grammatical mistake, "should be educated to specialists" is wrong - please correct this sentence. Same thing with line 394-395 - correct the sentence in these lines. Does not make sense to read it.

Response: 1) We have resentenced it to ‘We should raise an awareness of this practical point and pitfall among specialists involved.’ (Page 17, lines 415-416)

2) Line 394-395 was removed.

15. Authors should mention in their discussion section - importance of new born screening for early diagnosis of IOPD and obtain full benefit from early treatment with ERT.

Response: We add this message in the manuscript as follow: ‘Therefore, it is important that national newborn screening for early diagnosis of IOPD should be considered/implemented which could help the patients achieve full benefit from early treatment with ERT.’ (Page 17, lines 405-407)

16. Authors should also emphasize the importance of maintaining local mutation database along with CRIM and protein status so that future patients and treating physicians can benefit from this information.

Response: Thank you for this insightful advice.

We add this message in the discussion section. ‘It is also important that regional/local mutation database along with CRIM and protein status should be established and publicly accessible so that future patients and treating physicians can benefit from this information.’ (Page 18, lines 432-434)