Author’s response to reviews

Title: Genetic analysis of 62 Chinese families with Duchenne muscular dystrophy and strategies of prenatal diagnosis in a single center

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Author’s response to reviews:

Dear the editor,

Thank you for your letter of 6-June-2019, in which you informed us that our manuscript (MGTC-D-19-00119) may be considered further for publication but require revision according to the comments and suggestions of reviewers and editors.

We would like to thank the reviewers who critically reviewed the manuscript and gave insightful comments and suggestions, which were extremely helpful in improving our manuscript. We have revised our manuscript, taking into account the issues raised by the referees. Enclosed you will find our detailed responses to your specific concerns. Where appropriate, we have also indicated what steps have been taken to edit the manuscript.

As requested, we have revised our manuscript using red font instead of black to indicate the revised portions (the upload file named as 'revised manuscript'). With these changes, we believe that our manuscript has been improved substantially.

We sincerely hope our manuscript will be accepted to publication in BMC Medical Genetics. If there are any other problems about this manuscript, please not hesitate to contact us. We would be happy to
revise it to meet the standards of publication necessary. Thank you again and we await a favorable response to the revision. Please convey our thankfulness to the reviewers.

Sincerely yours,

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Response to Reviewers’ Comments
Reviewer: 1
1- The paper should be reviewed for the English by a native English speaker.
Response: Thanks. We have reviewed the English.

2- Authors should replace small mutation by point mutations
Response: Thanks for your comments. We were sorry for not accurately describing the mutations. We have replaced small mutations by point mutations.

3- In the methods, abstract section: authors should state briefly the type pf NGS performed (Panel, WES,...) and the used technology.
Response: Thanks for your suggestions. The inherited disease panel was used in the study. We have added the type of NGS in the abstract section (Page 2, line 40-43 red font).

4- In the results, abstract section: authors should add the mutations identified at the protein level. same section to replace "mothers showed the same mutations as the probands" by mothers were carriers for the same mutations as probands.
Response: Thanks for your suggestions. We have added the protein level of the mutations and replaced “mothers showed the same mutations as the probands” by “mothers showed positive results during carrier testing and de novo mutations arose in 7 probands” (Page 2, line48-51 red font).

5- authors should in the abstract precise that introns of DMD gene were not sequenced.
Response: We were sorry that we did not describe the introns of DMD gene not involved in our study. We have added it in the abstract (Page 2, line43 red font).

6- in the methods section: NGS design (primers design), number of samples per run, NGS analysis should be more detailed. authors describe this section in general. They should add more details.
Response: Thanks. According to the reviewer’s suggestion, we described the detailed the method of NGS analysis (Page 6, line 126-131, 139 red font).

7-results, line 149-150: the sentence "of all the exons, exon 49 and 50 ..... a history of DMD" is not clear.
Response: This sentence means that Exon 49 and 50 were the most frequently deleted exons in 32 families with exon rearrangements.
8- results line 164: why they screened for point mutations in the mothers by NGS not by fluorescent sequencing?
Response: We have corrected this mistake. Thank you very much (Page 8, line 166-167 red font).

9- discussion: authors didn't explain why molecular diagnosis for these 62 families was not performed before this study? at least MLPA which was available since many years.
Response: Because the members of 62 families did not perform the molecular diagnosis until the female members prepared for pregnancy, the molecular detection for the probands of 62 families were not performed before this study.

10- line 192-194: it could be more interesting to put some numbers when authors compare their results to other publications, instead of saying lower and higher than other studies.
Response: Thanks for your suggestions. We have added the numbers in the discussion (Page 9, line 196-197, 199 red font).

11-line 216: to replace new mutation by de novo mutation.
Response: Thanks. We have replaced “new mutation” by “de novo mutation” (Page 10, line 220 red font) .

12- line 223: to replace "and the remaining had no pathogenic mutation" by "the remaining were normal for the identified mutations in the families respectively"
Response: Thanks for your comments. We have corrected the sentence (Page 10, line228 red font).

13-did authors identified after studying the fetuses a family with germline mutation in mosaic state?
Response: That’s a good suggestion. We are sorry that we did not identify the family members with germline mutation in mosaic state. We could do the experiment in the future study.

14- why they didn't include the introns in their study?
Response: The commercial disease panel only detected the exons of the genes, so we did not include the introns of DMD gene in our study.

15- in the title authors mention "a strategy for prenatal diagnosis" , in all the paper authors didn't mention the needed time to perform the technique and the analysis.
Response: Thanks. We added the content in the discussion section (Page 11-12, line 235-238, 250-255 red font).

16- authors should change all their conclusion.
Response: We have changed the conclusion. Thanks (Page 11-12, line 250-255 red font).

17- it could be interesting to put some clinical info for the patients: age, clinical signs, CPK value. And also it can be interesting to compare the severity of the disease in patients with large rearrangements compared to patients with point mutations.
Response: Thanks for your comments. It would be very interesting to make this comparison. However, for the DMD family members coming to our center, our major task was to provide genetic diagnosis and prenatal diagnosis, while the DMD proband of the family had been diagnosed in other special hospitals. As a result, the clinical information of the proband was incomplete in our study, and the comparison between the clinical information and the mutation types was not available. Nevertheless, the information of the probands in the 6 families with novel mutations were successfully requested and supplied in the table2-revised.
18- to replace in the tables the codon stop X by *.
Response: We have corrected this mistake. Thank you very much.

Reviewer: 2
1- The authors say that single exon deletion are the most common type of deletion, however it is known that this kind of mutation can often be an artifact of the MLPA process (mutation in the probe annealing region), since they have primers for each exon, this should be corroborated.
Response: We are very sorry that in the origin manuscript we did not mention the experiment for verifying the single exon deletion detected by MLPA. For every case with single exon deletion, we all performed the RT-PCR to confirm the results of MLPA (Page 7, line 152 red font). If necessary, we can provide the results of RT-PCR.

2- There are samples that are negative for the test of DMD by MLPA and NGS. Since the authors used the "Ion AmpliSeq Inherited Disease Panel", have they checked other neuromuscular diseases in the panel?. If yes and still negative for the other diseases, it should be stated. If no, they should be checked.
Response: The inherited disease panel consists of 382 genes. These genes are associated with 700 inherited diseases according to NCBI ClinVar database. The diseases conclude other neuromuscular diseases. The detailed information is shown in the web: lifetechnologies.com/ampliseqready.

3- In Discussion in line 206 the statement "All the six novel nonsense mutations" is not true. There are four small deletions or insertions that led to premature stop codons and two nonsense mutations.
Response: We have replaced “all the six novel nonsense mutations” by “all the six novel mutations”. Thanks (Page 10, line 210 red font).

4- In Table 2 is written “Nonsence” instead of “Nonsense”. Anyway, this information are already included in table 1 and can be distinguished by using bold or other distinctive mark.
Response: We have corrected the spelling mistake (Table 2-revised). Thank you very much.

Reviewer: 3
1. In the abstract page 2 line 37 the word was should be included between study and aimed and the sentence reformulated as: "This retrospective study was aimed at supplying information on our 4-year clinical genetic and.......................
Response: Thanks for your comments. We have corrected the sentence (Page 2, line 37 red font).

2. The sentence in line 48 page 2 is difficult to understand: in 52 families with positive results, 45 mothers showed the same mutations as the probands? How could it be possible, that the proband had a different mutation from the mothers, this fact should be commented in results or discussion since it seems striking
Response: We are sorry for not describing the content exactly. We have replaced it by “In 52 families with positive results, 45 mothers (86.5%) showed positive results during carrier testing and de novo mutations arose in 7 probands” (Page 2, line 51-52 red font).

3. At the end of page 7, it is concluded that other molecular methods are requiered for those probands in 10 families with no positive findings of MLPA and NGS. This reviewer is assuming that the family has an history of DMD and therefore an index case, except if the "putative" index case is dead and there are no other cases in the family. Another possibility could be a mutation raised as "de novo
mutation" in the embryos. In any case these explanations should be included. Again, in page 8 lines 165 and 167 (table 1) 7 mothers out of 52 probands did not carry any mutation. For sure this are "de novo mutations appeared in the foetus, or in the first divisions of the zygote. If this is a possible explanation, please include it in the text.

Response: As the reviewer mentioned, some probands have been dead in the 62 families and there were no other patients in the family. According to the symptom described by the family members, we presumed that the proband was the DMD patient. Another reason is that we just detected the exons of DMD gene and not including the introns. Therefore, the clinical diagnosis should be firstly revised in the later research. If the manifestations and muscle biopsies were consistent with DMD, the introns of the DMD gene should be deeply investigated. If the introns of DMD gene were negative, the other muscle disease panels or whole-genome sequencing (WGS) could be used to analyze the pathogenic mutations. We have discussed them in the discussion section of the revised manuscript (Page 11, line 231-239 red font).

About “7 mothers of 52 probands did not carry any mutation.” We have discussed in the discussion section of origin manuscript (Page 10, line 221-225 red font).

4. In the discussion section, line 182 the sentence "next generation and strongly hope to determine" should be changed to "next generation and strongly wished to determine...."
Response: Thanks. We have corrected the sentence (Page 9, line 186 red font).

5. Discussion, first paragraph page 10. It should be clear a difference or distinction between the terms "de novo" mutation, which means a mutation appearing in the proband and not inherited, from "new mutations, unpublished so far in literature or in the databases". This difference is crucial and we believe that the novel mutations referred to in the first paragraph of page 10 are new mutations, not reported so far in literature.
Response: Thanks. We should differentiate the “de novo mutation” with “novel mutation”. We have corrected it in the revised manuscript.

6. Figure 2 showing the changes in the sequence of the novel mutations, should be composed in such a way that in addition to the sequence alignment, the point of the capillar chromatography, where the nucleotide change appear as well as the surrounding sequence is shown in a Sanger chromatogram. This way is more illustrative and easy to follow. ' In general a survey of English and editing of typos should be performed.
Response: Thanks for your comments. We added the figure of sanger sequencing in Figure 2-revised.