Title: Novel FOXL2 mutations in two Chinese families with blepharophimosis- ptosis- epicanthus inversus syndrome

Authors:

Min Xue (flyxuem@163.com)
Jie Zheng (174390607@qq.com)
Qing Zhou (369511550@qq.com)
Fielding J Hejtmancik (hejtmancikj@nei.nih.gov)
Yuan Wang (aydesm-1@163.com)
Shouling Li (shoulingli@126.com)

Version: 2
Date: 16 July 2015

Author's response to reviews: see over
Dear Prof. Sands,

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript. We appreciate editor and reviewers very much for your positive and enlightening comments and suggestions on our manuscript entitled “Novel FOXL2 mutations in two Chinese families with blepharophimosis-ptosis-epicanthus inversus syndrome”. (MS: 1629347721600907).

We have studied reviewer’s comments carefully and have made revision which marked in red in the paper. We have tried our best to revise our manuscript according to the comments. Attached please find the revised version, which we would like to submit for your kind consideration.

We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you.

Thank you and best regards.

Yours sincerely,

Min Xue

Corresponding author:

Name: Shouling Li

E-mail: shoulingli@126.com
List of Responses

Dear Editor and Reviewers,

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Novel FOXL2 mutations in two Chinese families with blepharophimosis-ptosis-epicanthus inversus syndrome” (MS: 1629347721600907). Those comments are all valuable and very helpful for revising and improving our paper, and have important guiding significance to our researches as well. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer’s comments are as flowing:

Responds to the reviewer’s comments:

Dear reviewer Laura Crisponi:

-Major Compulsory Revisions

1. Response to comment: The data presented in the manuscript must be better presented and debated in the discussion and emphasized in the conclusion.

Response: It is really true as reviewer commented that the indel mutation (c.675_690delinsT; p.A226_A230del) is the second FOXL2 poly-Ala contraction reported so far. So further discussion and conclusion are required for a deeper understanding on poly-Ala tract’s function and the phenotype influences of its contraction or expansion. In the revised manuscript, we have additionally discussed
the mechanism for the molecular pathogenesis and functional consequences of poly-Ala tract contraction comparing to the first deletion mutation, as suggested by reviewer. (The revised details can be found in line 186-209, page 9-10).

2. Response to comment: The clinical details of the female patients should be detailed.

Response: As the reviewer’s good advice, we have re-written this part (line 175-185) and added the female clinical date (see Table 1), including details of menstruation and pregnancies. Unfortunately, the hormone levels data are not available for they are not interested in the study of POF because there are no fertility problems in both families so far.

3. Response to comment: The background and the references list should be implemented.

Response: We have made correction according to the reviewer’s comments. According to recent findings, the background and the references list have been implemented with a comprehensive review of all the mutations found so far in FOXL2, including the proportion of all mutations and genotype-phenotype correlations. (The revised details can be found in line 73-85, page 4).

4. Response to comment: Results from pathogenicity prediction software should be reported into a table.

Response: Results from pathogenicity prediction software have been reported into table 2.

5. Response to comment: For the sequencing result reported in Figure 4 for the indel
mutation is not clear, the mutant electropherogram without alignment with the normal sequence should be added.

Response: The mutant electropherogram without alignment with the normal sequence have been added in figure 4.

6. Response to comment: Needs some language corrections before being published.

Response: We have checked the whole article carefully to improve the English writing and it’s my great honor to get your help on the language correction.

-Minor Essential Revisions

1. The gene name FOXL2 has been adjusted in italic throughout the text.

2. “polyalanine tract” has been added into keywords.

3. “BPES type II” has been adjusted to be used throughout the text.

4. “The” has been left out and the sentence starts with “Two”. (now change to line 47).

5. “helix” has been added to the “winged/forkhead transcription factor”. (now change to line 66).

6. FOXL2 has been specified protein (now change to line 67). Reference 7 (now change to reference 8) has been added to this sentence.

7. “deletion/insertion” has been substituted with “indel”. Space has been left after the second bracket. (now change to line 87).

8. The sentence has been revised to “Informed consent was obtained from all participated individuals or their guardians for research according to the tenets of the Declaration of Helsinki and Guidance of Sample Collection of Human Genetic
Diseases through the Ministry of Public Health of China
doesn’t have a specific page, please note this.

9. Table 1 has been removed.

10. In methods, only two sections: Patients and Mutational analysis (comprising PCR/PCR product cloning and Sequencing and Mutation Analysis) have been left.

11. In the section patients, some details on the probands have been specified, such as age, facial features et al. (The revised details can be found in line 99-104, page 5). In the section Mutational analysis, all living patients from the two families we performed the analysis on the entire FOXL2 gene. (The revised details can be found in line 136-137, page 7).

12. Both variations were further evaluated in family members and 100 normal controls. It has been adjusted. (now change to line 137).

13. “The” has been added before FOXL2 gene. (now change to line 154).

14. Pathogenicity prediction analysis has been done with mutation taster (see table 2).

15. Conclusions have been put in a separated section.

16. “granulose” has been changed in “granulosa”. (now change to line 169).

17. Reference “Moumné L, Fellous M, Veitia RA. Deletions in the polyAlanine-containing transcription factor FOXL2 lead to intranuclear aggregation. Hum Mol Genet. 2005 Dec; 14(23):3557-64.” has been added. (now change to line 195).

18. “poly-phenylalanine” has been corrected with “poly-Ala”. (now change to line 199,237).
19. Abbreviations: “poly-Ala tract” has been added for “poly-alanine tract”. The text has been adjusted accordingly.


21. The spaces have been revised throughout the manuscript, figures and figure legends.

- Discretionary Revisions

1. Figure 2 legend has been changed with “…two fragments of 304 and 289 bp using 6% agarose gel electrophoresis.”

2. Figure 3 legend has been changed with “Pictures representing the ocular defects of BPES patients from two Chinese families”.

From your comments, it’s obvious that you are an expert in this field, and there are lots of shortages in our study which need your instruction. At last, let me extend my sincere thanks to your suggestions and thank you so much for taking your valuable time to revise our paper.

Dear reviewer Hannah Verdin:

-Major Compulsory Revisions

1. Response to comment: The different studies describing the functional consequences
of the polyalanine tract should also be discussed in more detail and the study by Moumné et al. (Human Molecular Genetics 2005) should be added to discussion.

Response: It is really true as reviewer illustrated that the deletion of the polyalanine tract has never been described in BPES patient so far. So further discussion and conclusion are required for a deeper understanding on poly-Ala tract’s function and the phenotype influences of its contraction or expansion. In the revised manuscript, we have additionally discussed the functional consequences of the poly-Ala tract, especially the poly-Ala contraction, as suggested by reviewer. (The revised details can be found in line 186-209, page 9-10).

2. Response to comment: The discussion on the missense mutation could be more extensive.

Response: As the reviewer’s good advice, we have additionally discussed the results from pathogenicity prediction software and the phenotype of missense mutations inside and outside the forkhead domain. The results obtained from different pathogenicity prediction software suggest that this mutation may affect protein function. Taken together, these data testify that the functional alteration of FOXL2 transcription factor by the c. 223C>T substitution may be associated with BPES in this Chinese family. In our study, the BPES family carrying the p.Leu75Phe mutation has a typical clinical BPES phenotype, which further supports the possibility that the affected BPES individuals with missense mutation inside the forkhead domain might have a severe phenotype. (The revised details can be found in line 210-225, page 10-11).
-Minor Essential Revisions

1. The gene name FOXL2 has been adjusted in italic when the gene is meant and plain when the protein is meant throughout the text.

2. Space has been checked after round brackets, commas, … ).

3. The full name of FOXL2 has been corrected as “forkhead box L2”.

4. “p.L75F” has been changed to “p.Leu75Phe”.

5. All the affected family members of family A have the heterozygous indel mutation c.675_690delinsT. All the affected family members of family B have the heterozygous missense mutation c.223C>T. The mutations are absence in the unaffected members of both families. We have specified this in the revised version. (The revised details can be found in line 161-163, page8).

6. The mutations are absence in the 100 controls. We have specified this in the revised version. (The revised details can be found in line 161-163, page8).

7. “the” has been deleted before “Chinese families”. (now change to line 47, 86).

8. “ocular” has been replaced with “eyelid”. (now change to line 58, 59).

9. “granulose” has been corrected to “granulosa”. (now change to line 68).

10. A space has been inserted between “in” and “regulating”. (now change to line 70).

11. “mental retardation” has been changed to “intellectual disability”. (now change to line 143).

12. “gene” has been deleted after FOXL2. (now change to line 154).

13. The last comma has been replaced by “and”. (now change to line 159).

14. “putative” has been deleted. (now change to line 166).
We acknowledge your comments and suggestions very much, which are valuable in improving the quality of our manuscript. Special thanks to you for your good comments.

Dear reviewer Jeehyeon Bae:

Minor Essential Revisions

1. Response to comment: The order of Figures mentioned in the result has to be in a numeric order.
   Response: The order of Figures mentioned in the result has been in a numeric order in the revised version as reviewer suggested.

2. Response to comment: In Fig. 2, the labeled individual (II: 5) is not an affected personal. Please correct that.
   Response: We are very sorry for our incorrect writting. “II: 5” has been corrected to “II: 4”. Thank you sincerely for your careful review.

3. Response to comment: Authors have to address these mutations are heterozygous in the result section.
Response: The mutations are heterozygous have been addressed in the result section.

At last, thank you for your arduous work and instructive advices.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper. We appreciate for Editor/Reviewers’ hard work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.