Author’s response to reviews

Title: A Novel Small Deletion of LMX1B in a Large Chinese Family with Nail–Patella Syndrome

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Version: 1 Date: 19 Feb 2019

Author’s response to reviews:

Cover Letter for Revised Manuscript MGTC-D-18-00540

To: Dr. Victor Faundes, Editor

BMC Medical Genetics

Dear Dr. Victor Faundes,

We thank you very much for giving us an opportunity to revise our manuscript entitled "A Novel Small Deletion of LMX1B in a Large Chinese Family with Nail–Patella Syndrome" (reference ID: MGTC-D-18-00540), We express our sincere gratitude to the reviewers for their prompt, thorough scrutiny and positive recommendations towards improving the manuscript. We have undertaken a thorough revision on the manuscript taking into account every comment, suggestion and questions raised by the reviewers. A point-by-point response to the reviewer's comments and the details of changes are shown in the below.
We are pleased to submit our revised manuscript to BMC Medical Genetics for your publication consideration.

Thank you so much!

Best regards,

Faithfully,

JIN Fan, MD

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Editor Comments:

1. Variant should be interpreted according the ACMG 2015 guidelines

   Response: Thank you for this important suggestion. We have corrected the variant in our manuscript according the ACMG 2015 guidelines, i.e., ‘pathogenic’, ‘likely pathogenic’, ‘uncertain significance’, ‘likely benign’, and ‘benign’ to describe variants identified in Mendelian disorders”. In page 2, results, page 2 conclusion, and page 7, Bioinformatic analysis, we described the variant as "pathogenic mutation." All the corrections are highlighted in red.

2. Please show figures in a better resolution.

   Response: We have presented new figures with better resolution in the revised manuscript. We very much regret that the original figures we presented have a good resolution, but it seems not clear when transformed to PDF file. We are not sure what happened during this process and we would like present new figures if needed.
3. Please use the canonical transcript and protein sequences, and give the corresponding accession numbers in GenBank (formerly known as RefSeq).

Response: We have revised the canonical transcript and protein sequences, and added the accession number: NM_002316.3.

Reviewers’ comments:

Ralph Witzgall (Reviewer 1):

General comments

The authors describe a novel mutation in the LMX1B gene they detected in a Chinese five-generation-family suffering from nail-patella syndrome. Using molecular modeling they argue that the mutation leads to a loss of the DNA-binding activity of LMX1B. Besides that, the manuscript does not contain any additional information, in particular no functional data (such as electrophoretic mobility shift assay or luciferase reporter assay).

Response: We thank the reviewer for the comprehensive comments. Since the novel pathogenic variant LMX1B/c.712_714delTTC (p.Phe238del) occurred in the conserved homeodomain, it was predicted to affect DNA binding ability of the protein. We are going to verify its functional effect by using luciferase reporter assay or other methods in our next researches.

Major comments

(1) Figure 2. The authors only show the compound sequencing trace of the wild-type and mutant allele. This is difficult to interpret because the peaks from the two alleles overlap. The authors should subclone the PCR product of exon 4 and sequence the two alleles separately so that they do not miss any additional mutations.

I do not think that it is necessary to present the sequencing data of 4 patients.

Response: We accepted the reviewer’s suggestion, and removed the sequencing data of 4 patients from Figure 2. We have subcloned the PCR product of exon 4 and sequenced the two alleles separately. The results were show in Figures 2A and B in the revised manuscript. Thus, the figure legend for Figure 2 was re-written and DNA subclone was described in methods.
Minor comments

(1) Page 4, Background. I am not aware that the LMX1B protein consists of 406 amino acids, to
my knowledge it is 395 and 402 amino acids long, resp. (cf. reference 1).

Response: We thank the reviewer for the valuable comment. The LMX1B gene encode three
isoforms, isoform 1 (NM_002316.3, NP_002307.2) with 395 amino acids, isoform 2
(NM_001174147, NP_001167618) with 402 amino acids and isoform 3 (NM_001174146,
NP_001167617) with 406 amino acids long, respectively (https://www.ncbi.nlm.nih.gov
gene/4010). In our former manuscript, we presented the longest one (NP_001167617, isoform 3). Since the shortest one (isoform 1) is the predominant isoform, we have displaced isoform 3 (406 aa) with isoform 1 (395 aa) in our revised manuscript. The sentence in Page 4, Background, has been revised as "LMX1B comprises eight exons covering more than 90 kb and encodes the LIM homeobox transcription factor 1-beta protein, which consists of 395 amino acid residues."

(2) Page 7, Results, and Page 15, Legends to Figures 1 and 2. It should be "patient" and not "proband".

Response: We apologize for our slips of the pen. We have replaced "proband" with "patient".

(3) Page 8, Results. It either is homeobox or homeodomain, but not "homeobox domain". The
homeobox refers to the DNA sequence and the homeodomain to the protein sequence.

Response: Thank you so much for finding the error. We have since replaced "homeobox domain"
with "homeobox".

Takuya Takeichi (Reviewer 2)

1) Have the other in-frame deletions of LMX1B been reported in the other pedigree with nail-
patella syndrome in the literature? Please discuss this point in the paper.

Response: We thank the reviewer for this critical comment. To date, two causative in-frame
deletion mutations in other sites of LMX1B gene have been reported (ref 9 and ref 16). We have
discussed this issue in page 10.